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# Novel Room Temperature Ionic Liquid for Liquid-Phase Microextraction of Cannabidiol from Natural Cosmetics

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Received: 30 July 2020; Accepted: 24 August 2020; Published: 26 August 2020




**Abstract:** This study presents the synthesis of a novel asymmetric 1,3-di(alkoxy)imidazolium based room temperature ionic liquid, more precisely 1-butoxy-3-ethoxy-2-ethyl-imidazolium bis(trifluoromethane)sulfonimide, and its application as an extraction solvent in liquid-phase microextraction of cannabidiol from natural cosmetics. Quantification was implemented, using a high performance liquid chromatography system coupled to ultraviolet detection. Molecular structure elucidation was performed by nuclear magnetic resonance spectroscopy. The extraction procedure was optimized by means of two different design of experiments. Additionally, a full validation was executed. The established calibration model, ranging from 0.6 to 6.0 mg $g^{-1}$, was linear with a coefficient of determination of 0.9993. Accuracy and precision were demonstrated on four consecutive days with a bias within −2.6 to 2.3% and a maximum relative standard deviation value of 2.5%. Recoveries, tested for low and high concentration within the calibration range, were 80%. Stability of extracted cannabidiol was proven for three days at room temperature and fourteen days at 4 °C and −20 °C. An autosampler stability for 24 h was validated. Liquid-phase microextraction of cannabidiol from different formulated cream based cosmetics was performed, including four ointments and four creams. The results show that a significantly higher selectivity could be achieved compared to a conventional extraction methods with methanol.

**Keywords:** room temperature ionic liquid; cannabidiol; liquid-phase microextraction; cosmetic

## 1. Introduction

Analytical sample preparation is an essential step in modern analysis. Its purpose is to isolate analytes of interest from complex matrices or interfering chemicals. This ensures a satisfying determination by guaranteeing selectivity, accuracy, reproducibility, and reliability of analysis. [1,2]

Various techniques for extraction and isolation of different types of analytes were developed over the last decades, particularly in the field of liquid–liquid and solid-phase extraction. Liquid–liquid extraction (LLE) is one of the oldest extraction procedures in analytical chemistry, in which the analytes are transferred from a liquid sample into an immiscible solvent. However, LLE is a time-consuming and environmentally unfriendly extraction process, due to large solvent consumption [3–5].

To overcome those disadvantages, a more suitable and beneficial sample preparation procedure, so called liquid-phase microextraction (LPME) or liquid–liquid microextraction (LLME), has attracted more and more attention over the past years. LPME has the advantage, that the solvent amount is reduced to the microliter range. In some applications, even a single drop is sufficient [4–6]. Recently, several LPME procedures for different applications, using ionic liquids (ILs) as extraction solvent, have been developed [7–15].

ILs are defined as salts with a melting point below one hundred degree Celsius. So called room temperature ionic liquids (RTILs) are a sub-category and have a melting point below twenty five degree Celsius [16]. The two main characteristics of ILs are the low melting point and the ionic conductivity. Moreover, many ILs exhibit other unique physicochemical properties, such as low vapor pressure, thermal and electrochemical stability, no flammability, as well as the potential to dissolve both organic and inorganic substances [17–19]. ILs can be designed particularly for specific applications, since their physiochemical properties strongly depend on the composition of the salt [20,21].

Cannabidiol (CBD) is one of the major cannabinoids, the characteristic components of the plant *Cannabis*. CBD is non-psychoactive and has several positive effects on health issues, due to its anti-inflammatory, anti-oxidative, and anti-necrotic protective effects. It is commonly used for neurological diseases, such as epilepsy, Alzheimer's disease, Parkinson's disease, as well as anxiety disorder [22]. For several years, the positive dermatologic effects of CBD have been causing increased interest. It can be used as treatment for acne vulgaris, skin eczema and dermatitis, pruritus, psoriasis, and skin cancer [23]. As a consequence of the "CBD-boom", numerous CBD cosmetics from many different suppliers are now available on the free market. To accurately measure and control the CBD content in those products, reliable quantifications are highly necessary. Due to the strong matrix effects, resulting from cream base and other interfering compounds, efficient sample preparation techniques have to be developed [24].

The present study introduces the synthesis of a novel, highly apolar and pure room temperature ionic liquid (RTIL) consisting of an asymmetric 1,3-di(alkoxy)imidazolium cation and a triflimidate anion, more precisely 1-butoxy-3-ethoxy-2-ethyl-imidazolium bis(trifluoromethane)sulfonimide [BuEtEtIMO] [$Tf_2N$]. This RTIL was used to develop an efficient and robust liquid-phase microextraction technique in conjunction to high performance liquid chromatography coupled to ultraviolet detection (HPLC–UV), to accurately quantitate the CBD content in natural cosmetics. The workflow was optimized using two different design of experiments (DoE) to investigate categorical and numerical factors, respectively. After the optimization, the procedure was validated according to the guidelines of the "Society of Toxicological and Forensic Chemistry" (GTFCh) including selectivity, linearity, limit of detection, limit of quantification, accuracy and precision, as well as recovery and stability.

## 2. Materials and Methods

### *2.1. Reagents and Materials*

Glyoxal (40% in $H_2O$), propionaldehyde (≥97%), hydrochloric acid (HCl ≥ 37%), potassium hydroxide (KOH 90%), diethyl sulfate ($Et_2SO_4$ ≥ 99%), 1-bromobutane (99%), and lithium bis(trifluoromethane)sulfonimide ($LiTf_2N$ 99%) were all purchased from Sigma Aldrich (Buchs, Switzerland). The solvents methanol (MeOH), acetonitrile (ACN) and dichloromethane (DCM) were all HPLC grade (Chromasolv) from VWR (Radnor, PA, USA). Sodium hydroxide solution (NaOH ≥ 32%) and formic acid (FA ≥ 98%) were obtained from Carl Roth (Karlsruhe, Germany). Hydroxylamine hydrochloride (99%) was purchased from Alfa Aesar (Haverhill, MA, USA), activated carbon from Fluka (Hampton, NH, USA) and cannabidiol (CBD, Reference Material–Primary Standard) from LGC (Teddington, UK). Water was obtained from a Millipore Milli-Q water purification system (Bedford, MA, USA). A standard mix solution (1000 µg $mL^{-1}$) was prepared in MeOH. The stock solution was stored at −20 °C in the dark. Working standard solutions were daily prepared by diluting the stock solution with ACN.

### 2.2. Instrumentation

Analyses were performed using a 1100 Series HPLC Value System from Agilent (Santa Clara, CA, USA) equipped with a 1100 Series diode array detector (DAD). Separation was executed using a Luna 3u C18(2) (100 Å, 2.0 × 100 mm, Phenomenex, Torrance, CA, USA) analytical column. Mobile phase was composed of formic acid 0.1% (*v/v*) in water (eluent A) and formic acid 0.1% (*v/v*) in ACN (eluent B). Elution was performed isocratic with 70% (*v/v*) of eluent B at a flow rate of 0.7 mL $min^{-1}$. Injection volume was 10 µL. Temperature of the column oven was set to 40 °C and detection was performed at 228 nm. NMR experiments for molecular structure elucidation of the novel RTIL was conducted on a 400 MHz Bruker Avance 4 Neo spectrometer (2 channels, rt BBFO probe). For IL synthesis a Yellow Line MSC basic C magnetic stirrer from IKA (Staufen im Breisgau, Germany), a 300 series rotavapor from Buchi (Flawil, Switzerland) and a centrifuge Z 400 K from Hermle (Gosheim, Germany) were used. LPME experiments were performed on an Ultrasonic Cleaner USC—TH from VWR (Radnor, PA, USA) and on a ThermoMixer C from Eppendorf (Hamburg, Germany). Separation of solution and residual cream was carried out on a Centrifuge 5418 from Eppendorf (Hamburg, Germany). Evaporation of solvents was performed using a Concentrator plus from Eppendorf (Hamburg, Germany) and an Eva from VLM (Bielefeld, Germany).

### 2.3. Synthesis of Ionic Liquid

#### 2.3.1. 1-Hydroxy-2-ethyl-imidazol-3-oxide (**1**)

The synthesis of the basic structure 1-hydroxy-2-ethyl-imidazol-3-oxid [HEtIMO] (Figure 1, **1**) was performed according to a modified protocol, based on an already known synthesis [25,26]. For its implementation, the reaction of glyoxal, hydroxylamine hydrochloride and propionaldehyde was executed in an acidic environment. Glyoxal (40%, 50.0 g, 0.34 mol) and propionaldehyde (97%, 24.3 g, 0.41 mol) was mixed with 77 mL MeOH. The reaction mixture was stirred and cooled using an ice-ethanol bath. Meanwhile, a hydroxylamine hydrochloride (99%, 48.4 g, 0.69 mol) solution in 55 mL water was prepared. Subsequently, the solution was added dropwise to the constantly cooled glyoxal-propionaldehyde mixture. After removing the ice-ethanol bath, HCl (37%, 7 mL, 0.08 mol) was added dropwise to the reaction mixture. Stirring the solution at room temperature was continued for another 19 h. After the time had expired, the reaction mixture was cooled with an ice-ethanol bath. A NaOH-solution (32%, 70 mL, 0.76 mol) was added dropwise, whereby a white solid precipitated. The precipitation was filtered off and washed with ice-cold MeOH (100 mL). The resulting product was dried in a vacuum drying oven (30 °C, 50 mbar, 24 h) to gain pure [HEtIMO] as a white powder. Yield: 36.7 g (83.1%)—$^1$H NMR (400 MHz, $CDCl_3$): δ = 1.09–1.13 (t, *J* = 7.70 Hz, 3H), 2.63–2.69 (q, *J* = 7.60 Hz, 2H), 7.19 (s, 2H) (spectrum is illustrated in Figure S1).

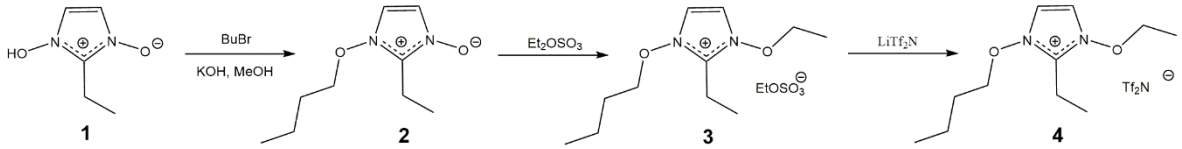



**Figure 1.** Synthesis scheme of [BuEtEtIMO] [$Tf_2N$].

#### 2.3.2. 1-Butoxy-2-ethyl-imidazol-3-oxide (**2**)

1-Butoxy-2-ethyl-imidazol-3-oxide [BuEtIMO] (Figure 1, **2**) was obtained through a nucleophilic substitution with 1-bromobutane by modifying already published protocols [26,27]. In the first step, KOH (90%, 27.9 g, 0.45 mol) was dissolved in MeOH (99%, 220 mL). The obtained solution was combined with [HEtIMO] (44.1 g, 0.34 mol) under stirring. In the next step, 1-bromobutane (99%, 47.5 g, 0.34 mol) was added dropwise. After the complete addition, the reaction mixture was stirred at 50 °C under reflux for 22 h. In the next step, the mixture was cooled with an ice-ethanol bath.

After filtering off the precipitate, the solvent was evaporated. Subsequently, DCM (100 mL) was added for further precipitation. The suspension was cooled with an ice-ethanol bath, before filtering off the remaining solid. Then the solvent was evaporated. The steps, starting with the addition of DCM, were repeated until no further precipitation was visible. In the last step [BuEtIMO] was obtained as a brownish to black, viscous liquid. Yield: 51.2 g (80.7%)—$^1$H NMR (400 MHz, [$D_6$]DMSO): δ = 0.91–0.95 (t, *J* = 7.42 Hz, 3H), 1.14–1.18 (t, *J* = 7.59, 3H), 1.38–1.47 (m, 2H), 1.63–1.70 (m, 2H), 2.74–2.80 (q, *J* = 7.48, 2H), 4.22–4.25 (t, *J* = 6.52, 2H), 7.07–7.08 (d, *J* = 2.27, 1H), 7.56–7.57 (d, *J* = 2.24, 1H) (spectrum is illustrated in Figure S2).

#### 2.3.3. 1-Butoxy-3-ethoxy-2-ethyl-imidazolium ethyl sulfate (**3**)

The synthesis of 1-butoxy-3-ethoxy-2-ethyl-imidazolium ethyl sulfate [BuEtEtIMO] [$EtOSO_3$] (Figure 1, **3**) was accomplished, based on two descriptions derived from literature [26,27]. Diethyl sulfate (99%, 43.4 g, 0.28 mol) was added dropwise to [BuEtIMO] (51.2 g, 0.28 mol), while cooling with an ice bath. Subsequently, the ice bath was removed and the reaction mixture was stirred for another 20 h at room temperature. In the next step, the gained brown liquid had to be purified. For this purpose, the reaction mixture was diluted with water (80 mL) and the water phase extracted with DCM (80 mL) twice. The water phase was separated and further purified by adding activated carbon (2 g). The suspension was shaken overnight on a thermomixer (30 °C, 1000 rpm). Subsequently, the solid was filtered off and the activated carbon step repeated until the water phase was colorless.

#### 2.3.4. 1-Butoxy-3-ethoxy-2-ethyl-imidazolium bis(trifluoromethane)sulfonimide (**4**)

$LiTf_2N$ (15 g) was dissolved in water (15 mL) to obtain a 1 g $mL^{-1}$ $LiTf_2N$-solution for salt metathesis. Therefore, the prepared solution was added to the purified [BuEtEtIMO] [$EtOSO_3$] in water, whereby a colorless second phase was formed. During the salt metathesis the reaction vessel was repeatedly shaken on a thermomixer (2–5 min, 1000 rpm). The addition of $LiTf_2N$ solution was pursued until no further turbidity and phase separation could be observed. Finally, the mixture was centrifuged (25 °C, 3500 rpm), the aqueous supernatant discarded, and the residual IL dried for 24 h in a vacuum centrifuge. The product [BuEtEtIMO] [$Tf_2N$] (Figure 1, **4**) was a slightly yellowish, viscous liquid. Yield: 6.2 g (6.5%)—$^1$H NMR (400 MHz, [$D_6$]DMSO): δ = 0.94–0.98 (t, *J* = 7.45, 3H), 1.32–1.28 (t, *J* = 7.67, 3H), 1.35–1.39 (t, *J* = 7.02, 3H), 1.41–1.50 (m, 2H), 1.71–1.79 (m, 2H), 3.02–3.08 (q, *J* = 7.57, 2H), 4.42–4.45 (t, *J* = 6.58, 2H), 4.46–4.51 (q, *J* = 7.05, 2H), 8.24–8.26 (m, 2H) (spectrum is illustrated in Figure S3).

#### 2.3.5. 1-Butoxy-3-methoxyimidazolium bis(trifluoromethane)sulfonimide

1-Butoxy-3-methoxyimidazolium $Tf_2N$ [BuMeIMO] [$Tf_2N$] was synthesized as previously described by Meischl, Harder et al. (2019) [26].

### *2.4. Liquid-Phase Microextraction Experiments*

Two different DoE approaches using the software Design-Expert® Version 10 (Stat-Ease, Inc., Minneapolis, United States) were performed to develop an efficient RTIL based LPME method for the isolation of CBD from cosmetics. In the first step, a minor screening design for the determination and selection of the categorical parameters was conducted. Subsequently, a full central composite design was carried out to investigate and optimize the numerical parameters of the extraction.

#### 2.4.1. Screening Design for Categorical Parameters

For the investigation and selection of categorical parameters, in regard to a high extraction recov-ery, a simple screening design was performed. Examined parameters were: type of RTIL ([BuMeIMO] [$Tf_2N$] versus [BuEtEtIMO] [$Tf_2N$]), type of extraction (thermomixer versus ultrasonic bath), as well as type of evaporation (SpeedVac Concentrator versus evaporation under nitrogen-stream)

(Plackett-Burman table is illustrated in Table S1). A salve based on an oil-in-water emulsion formulation containing 6 mg CBD per g salve (abbreviated mg $g^{-1}$), was used for all experiments. In the first step, 20 mg of cream were weighed in an Eppendorf vial and 1 mL of MeOH was added. The mixture was sonicated for 10 min to receive a complete suspension. Subsequently, 20 µL of respective RTIL was added. The actual extraction was conducted either in a temperature controlled ultrasonic bath (<40 °C) or on a ThermoMixer (1800 rpm, 25 °C) for 60 min. Afterwards, the residual cream was separated by centrifugation with device settings of 14,000 rpm and 10 min. The supernatant was evaporated either under nitrogen-stream or applying a SpeedVac Concentrator. The remaining RTIL was diluted with ACN (1/29, *v/v*) and measured using a HPLC–UV system.

#### 2.4.2. Full Central Composite Design for Numerical Parameters

In the next step, a DoE approach using a full central composite design with six center points and an alpha value of 1.5 was performed, to investigate and optimize the numerical parameters of the LPME procedure. The examined factors were: volume of RTIL and MeOH, amount of cream as well as extraction time. The applied levels are listed in Table 1. A total of four responses were included into the design, comprising of extraction recovery values of four different CBD containing creams. The samples were two salves with an oil-in-water emulsion formulation containing 6 and 1.25 mg $g^{-1}$, respectively, as well as two water-in-oil emulsion formulations. One of the water-in-oil emulsion samples was already purchased as a CBD-cosmetic with a concentration of 3 mg $g^{-1}$. The second water-in-oil emulsion was spiked with CBD to obtain a concentration of 0.8 mg $g^{-1}$. For the experimental implementation, a certain amount of cream was weighed in an Eppendorf vial. Subsequently, respective volumes of MeOH and RTIL were added, using a pipette. In the next step, the suspension was set in a temperature controlled ultrasonic bath (<40 °C). After the respective time had expired, the cream residual was separated by centrifugation at 14,000 rpm for 10 min. The supernatant was evaporated by a SpeedVac Concentrator. The remaining RTIL was diluted with ACN (1/29, *v/v*) and the solution measured on a HPLC–UV system.

**Table 1.** Parameter levels for the full central composite design.

| Parameter | Level −1.5 (−α) | Level −1 | Level 0 | Level +1 | Level +1.5 (+α) |
|---|---|---|---|---|---|
| amount of cream/mg | 12.5 | 15.0 | 20.0 | 25.0 | 27.5 |
| volume of MeOH/mL | 0.25 | 0.5 | 1.0 | 1.5 | 1.75 |
| volume of IL/µL | 5.0 | 10.0 | 20.0 | 30.0 | 35.0 |
| extraction time/h | 0.25 | 0.5 | 1.0 | 1.5 | 1.75 |

#### 2.4.3. Optimized Liquid-Phase Microextraction Procedure

With the obtained results from DoE experiments (see in Section 3.1.2), an optimized LPME procedure could be developed according to the following steps. Initially, 15 mg of cream was weighed in an Eppendorf vial. Additionally, 0.5 mL MeOH and 30 µL RTIL was added. The suspension was sonicated for 30 min, with a controlled maximum temperature of 40 °C. Afterwards, the residual salve was separated by centrifugation at 14,000 rpm for 10 min. The supernatant was evaporated by a SpeedVac Concentrator. The remaining RTIL was diluted with ACN (1/29, *v/v*) and the solution measured on a HPLC–UV system.

#### 2.4.4. Method Validation

To guarantee a reliable and replicable method for the quantification of CBD in creams, method validation was performed. The validation of the LPME procedure, followed by HPLC analysis, was executed according to the international guidelines of the "Society of Toxicological and Forensic Chemistry" (GTFCh) with minor adjustments [28–30]. The investigated validation criteria included selectivity, linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy and precision, as well as recovery and stability.

Selectivity was determined within two steps. At first, six blank matrix samples purchased from different companies, were proceeded and checked for interferences with regard to the analyte peak. In the next step, the developed LPME procedure was compared to the same procedure using only pure MeOH as extractant.

For the determination of limit of detection (LOD) and limit of quantification (LOQ), seven CBD concentration levels between 0.06 and 0.6 mg $g^{-1}$ (0.06, 0.15, 0.3, 0.45, 0.6, 0.75, and 0.9 mg $g^{-1}$) were prepared. Therefore, blank matrix extracts containing no analyte, were spiked with standard solution. All concentration levels were prepared and measured three times, to obtain a total of nine measurement results for each level. LOD and LOQ were calculated according to german industry norm DIN32645: 2008-11.

A calibration model was established in relation to the common CBD amount in purchasable products, hence the lowest calibration point was higher than the determined LOQ. The five CBD concentration levels range from 0.6 to 6.0 mg $g^{-1}$ (0.6, 1.5, 3.0, 4.5, and 6.0 mg $g^{-1}$). For the practical implementation, five blank matrix extracts were spiked with standard solution. All CBD concentration levels were prepared and measured three times. In order to determine the linearity of the calibration model, coefficient of determination, bias and relative standard deviation (RSD) for each calibration level, were calculated.

To investigate the extraction recovery of CBD, ten quality control (QC) samples, five at low (QC1, 0.6 mg $g^{-1}$) and five at high (QC2, 4.5 mg $g^{-1}$) concentrations, were prepared and proceeded (LPME implemented as described in Section 2.4.3). As reference material three blank matrix extracts were spiked with standard solution to get respective concentration levels. The recovery was determined by comparing the absolute peak area of QC samples with the absolute peak area of the reference material.

To demonstrate accuracy and precision, as well as repeatability on four consecutive days, three QC samples at low (QC1, 0.6 mg $g^{-1}$) and three QC samples at high (QC2, 4.5 mg $g^{-1}$) concentrations were proceeded. The measured concentrations were multiplied with the correction factor of 1.25 to compensate the losses of extraction (see in Section 3.2.3). Bias and RSD were calculated for each day (intraday) and for the entire period (interday).

Stability of extracted CBD in undiluted RTIL at three different storage temperatures (−20, 4, and 25 °C) as well as autosampler stability was examined. Therefore, QC samples at low (QC1, 0.6 mg $g^{-1}$) and high (QC2, 4.5 mg $g^{-1}$) concentrations were prepared and processed. The RTIL-extracts (LPME implemented as described in 2.4.3) with equal concentrations, were pooled and the aliquots stored at respective temperatures. Three aliquots of each concentration were diluted and measured immediately, to obtain reference values. After one, three, seven and fourteen days, three aliquots of each concentration and storage temperature were diluted and measured. Additionally, for the proof of autosampler stability processed QC samples at low (QC1, 0.6 mg $g^{-1}$) and high (QC2, 4.5 mg $g^{-1}$) concentrations were measured over 24 h. Stability was given, if the obtained absolute peak area did not deviate more than 10% from the initial value [28].

#### 2.4.5. Real-World Samples

The study was completed with the determination of eight real-world samples, which were acquired in seven different local stores and Austrian web shops. The products included four salves with an oil-in-water emulsion formulation and four creams with a water-in-oil emulsion formulation). The main ingredients distinguish between all salves, even though the solid consistence is nearly the same. The main component of the creams is water, therefore the consistence is soft and moist. The ingredient list of all real-world samples are summarized in Table S2. All real-world samples were worked up according to the optimized LPME procedure, described in Section 2.4.3. The measured concentrations were multiplied with the correction factor of 1.25 to compensate the losses of extraction (see in Section 3.2.3).

## 3. Results

### *3.1. Optimization*

#### 3.1.1. Screening Design for Categorical Parameters

LPME method development is essential to achieve high and reproducible extraction recoveries, along with minimum time and effort. For that purpose, twor DoE approaches were performed, using the software Design Expert® Version 10. At first, a screening experiment was executed, to determine which categorical factors have a significant influence on the extraction recovery and which factor are most suitable regarding practical work. It was observed, that only the type of RTIL had a significant effect considering the Bonferroni limit (see Pareto Chart in Figure S4). Type of extraction and type of evaporation had no significant influence on extraction recoveries. [BuEtEtIMO] [$Tf_2N$] showed much higher recoveries than [BuEtIMO] [$Tf_2N$] and was, therefore, chosen for further experiments. SpeedVac Concentrator was selected for solvent evaporation, due to the slight centrifugation power, which helped to accumulate the RTIL phase on the bottom of the vial. For the extraction type, sonication was the more suitable way, hence the first sonicating-step of accomplishing a salve-MeOH suspension, could be skipped.

#### 3.1.2. Full Central Composite Design for Numerical Parameters

In order to determine, if a parameter or interactions of parameters have a significant influence on the response factors, an analysis of variance (ANOVA), was executed using the software Design Expert® Version 10. ANOVA results of the main effects for linear and quadratic models are provided in the Table S3. The results for both models, showed two comprehensive significant parameters, including volume of RTIL and MeOH. Amount of cream was observed to be significant for three out of four responses. Extraction time was a non-significant parameter and had no effect on the responses. The results of the quadratic model demonstrated, that all parameter interactions had no significant effect on the responses. The most fitting procedure for RTIL assisted LPME of CBD from cosmetics was established using the tool optimization. Aim of the optimization was to find certain values for all numerical parameters to maximize the recovery of CBD for all four different creams. The highest desirability for the linear model (0.932), could be obtained by using the following experimental settings: 15 mg of cream, 0.5 mL of MeOH, 30 µL RTIL, and 0.5 h extraction time. Using the quadratic model, the highest desirability (0.906) was given with 17.3 mg of cream, 0.5 mL of MeOH, 30 µL RTIL, and 1.5 h extraction time. The optimized parameters from the linear model were chosen for further experiments, due to practically compliance of the optimal setting for the three significant factors.

### *3.2. Method Validation*

#### 3.2.1. Selectivity

The selectivity was verified, since no interferences with the analyte CBD were observed in all proceeded blank matrix samples. A comparison between the novel RTIL as extracting agent and a common pure MeOH extract, showed significant better selectivity towards CBD by using RTIL assisted LPME (illustrated in Figure 2).

#### 3.2.2. LOD, LOQ and Linearity

The obtained values for LOD and LOQ were 0.01 and 0.03 mg $g^{-1}$, respectively. Validation parameters of the measurements for determination of LOD and LOQ are summarized in Table S4. The calibration model ranging from 0.6 to 6.0 mg $g^{-1}$ with five calibration levels, showed a good linearity with a coefficient of determination of 0.9993. Values were within the validation limits of ± 5% and 2.5% for bias and RSD, respectively. All results are summarized in Table 2.

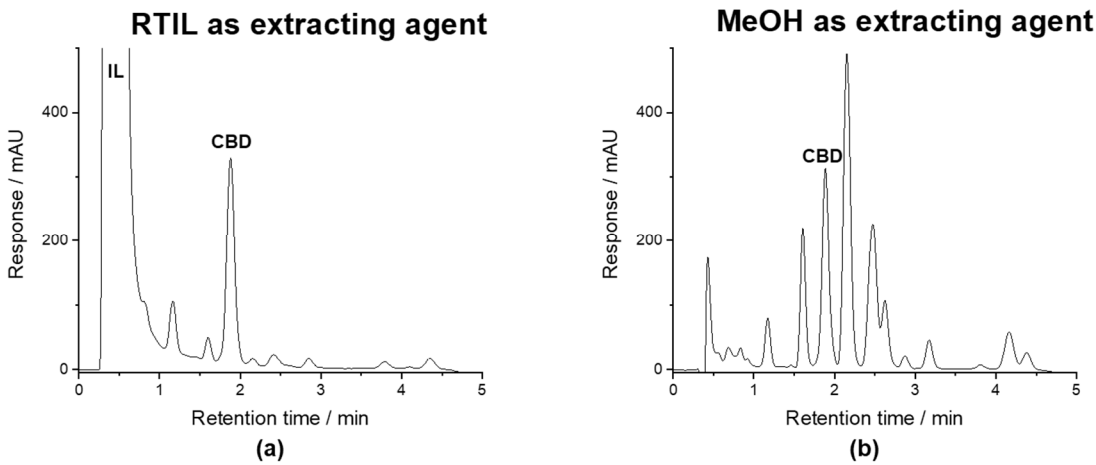



**Figure 2.** Room temperature ionic liquid (RTIL) as extracting agent (**a**) in comparison to a microextraction with pure MeOH (**b**) for the isolation of cannabidiol (CBD).

**Table 2.** Validation parameters for the linear model.

| Calibration Level/mg $g^{-1}$ | Bias/% | RSD/% |
|---|---|---|
| 0.6 | 1.8 | 1.1 |
| 1.5 | −2.4 | 1.3 |
| 3.0 | 0.5 | 1.7 |
| 4.5 | 0.9 | 0.9 |
| 6.0 | −0.5 | 1.3 |

### 3.2.3. Recovery

Extraction recoveries were close to 80% with RSD values lower than 2.5% for low as well as high concentrations (see Figure 3). The reference material was a RTIL-extract of blank cream spiked with a certain amount of CBD. This is in accordance to the validation criteria of a minimum recovery of 50%. In compensation of the lower recovery a correction factor of 1.25 was introduced, which led to a satisfying determination of accuracy and precision (see Section 3.2.4).

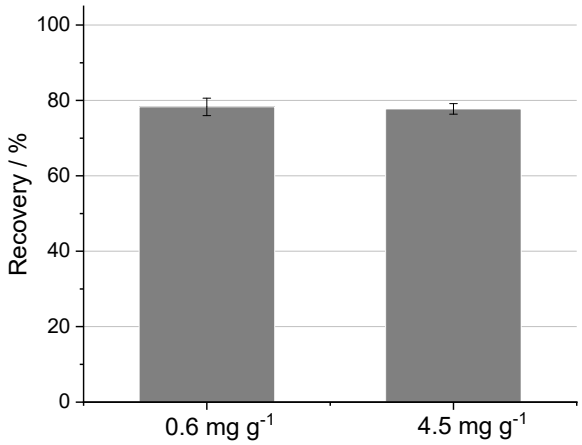



**Figure 3.** Recovery rates at low (0.6 mg $g^{-1}$) and high (4.5 mg $g^{-1}$) concentration.

### 3.2.4. Accuracy and Precision

Accuracy and precision (intraday as well as interday) were demonstrated on four consecutive days with two QC levels. Bias values were between −2.6 to 2.3% and the maximum RSD value was 2.5%. The detailed bias and RSD values are summarized in Table 3.

**Table 3.** Bias and relative standard deviation (RSD) values for determination of accuracy and precision.

| | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 1–4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bias [1] | RSD [1] | Bias [1] | RSD [1] | Bias [1] | RSD [1] | Bias [1] | RSD [1] | Bias [1] | RSD [1] |
| QC1 (0.6 mg $g^{-1}$) | 2.3 | 1.6 | −2.6 | 1.3 | −1.6 | 1.9 | 0.1 | 2.1 | 0.4 | 2.5 |
| QC2 (4.5 mg $g^{-1}$) | 1.5 | 2.4 | −0.8 | 2.2 | 1.0 | 1.7 | 1.6 | 1.9 | 0.8 | 2.2 |

[1] bias and RSD values in %.

### 3.2.5. Stability

Storage stability of CBD in undiluted RTIL at −20 °C and 4 °C could be verified over a period of fourteen days, due to a maximum decrease of 9.0% of the absolute peak area. Stability at 25 °C was only given for three days, since a higher decrease of absolute peak area after seven days was observed. Stability values are illustrated in Figure 4. CBD was found to be stable in the autosampler for at least 24 h. The results showed, that a storage of extracted CBD in pure RTIL over a period of 14 days at 4 °C and analysis overnight are acceptable.

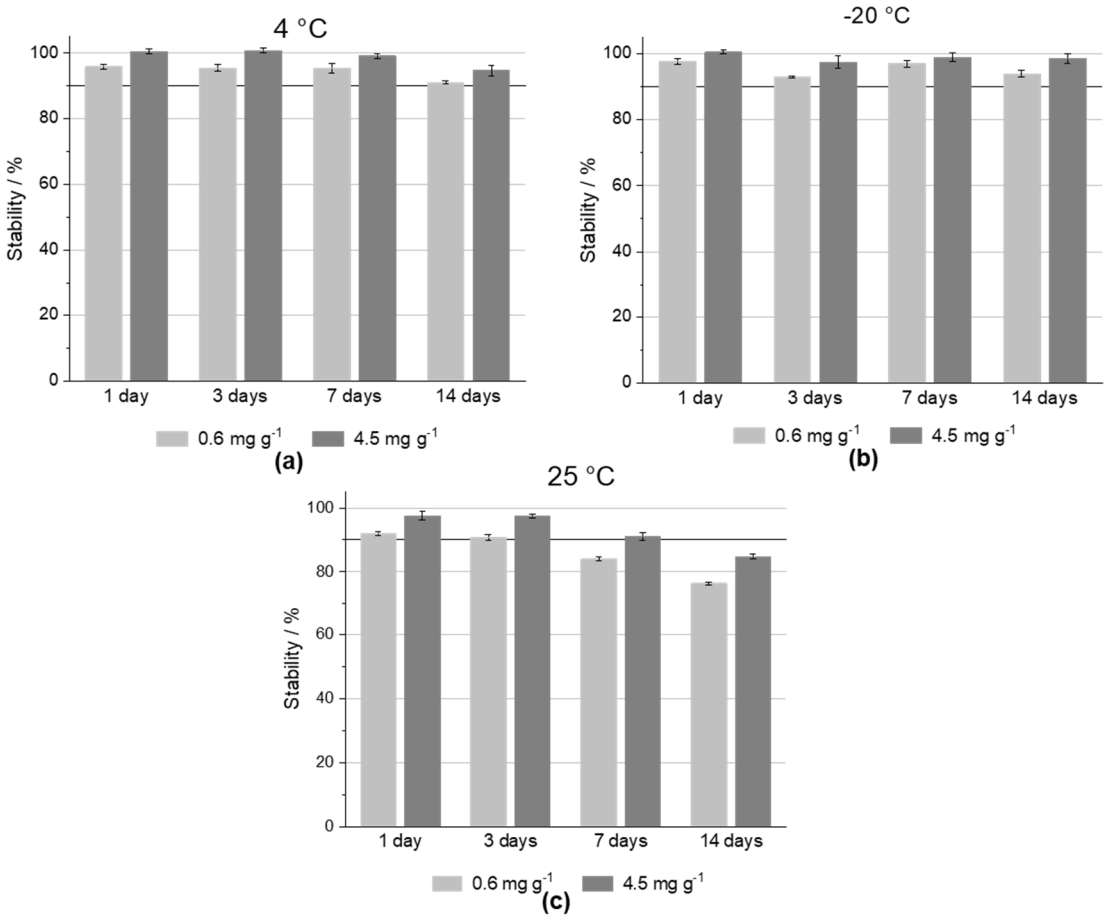



**Figure 4.** Stability of CBD in the RTIL extract (**a**) at 25 °C, (**b**) 4 °C, and (**c**) −20 °C.

## *3.3. Real-World Samples*

The developed and validated extraction procedure was used for the determination of CBD in eight different real-world samples, including four ointments and four creams. The chromatogram obtained from the analysis of an oil-in-water emulsion is illustrated in Figure 5. Five samples showed, an amount of CBD higher than 0.6 mg $g^{-1}$. In one sample CBD could be detected, though the concentration was between LOD and LOQ. In two other samples CBD could not be detected. All results are summarized in Table 4.

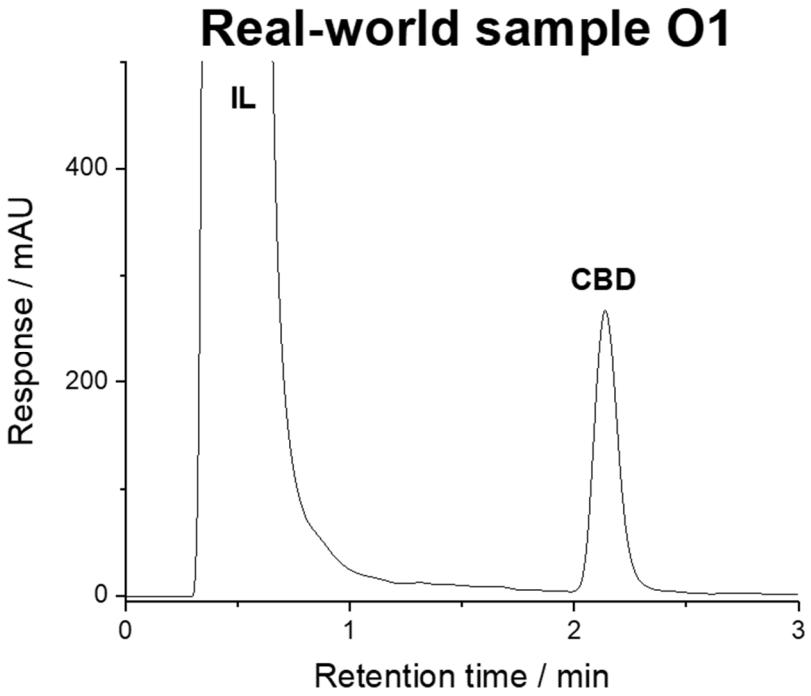



**Figure 5.** Chromatogram of an analysis of one real-world sample with an oil-in-water emulsion.

**Table 4.** Results of real-world samples.

| Formulation | Product No. | Nominal Concentration of CBD | Measured Concentration of CBD (RSD) |
|---|---|---|---|
| oil-in-water emulsion | O1 | 6.0 mg $mL^{-1}$ | 4.61 mg $g^{-1}$ (1.4%) |
| | O2 | 0.3% | 1.44 mg $g^{-1}$ (0.8%) |
| | O3 | 1.25 mg $mL^{-1}$ | 0.95 mg $g^{-1}$ (1.6%) |
| | O4 | unspecified | <LOQ |
| water-in-oil emulsion | W1 | 3.0 mg $mL^{-1}$ | 1.81 mg $g^{-1}$ (0.9%) |
| | W2 | unspecified | 0.87 mg $g^{-1}$ (2.1%) |
| | W3 | unspecified | n.d.[1] |
| | W4 | unspecified | n.d.[1] |

[1] not detected.

The measured CBD concentrations were all lower than the declared concentrations on the products list of ingredients. There are several reasonable explanations for this issue. One possibility is, that CBD is not completely stable in cosmetics and its concentration decreases during the transportation and storage. In some creams CBD is additionally added to the formulation, however dosage form and purity are not indicated on the product. Therefore, a second explanation could be that the added CBD is of less purity and consequently a wrong CBD content was calculated by the manufacturers. The density of creams and ointments is around 0.9 g $ml^{-1}$. The different units (mg $mL^{-1}$ vs. mg $g^{-1}$) have only a small effect on the results and are therefore not responsible for the lower concentrations.

## 4. Discussion and Conclusions

The present study describes the synthesis and structure elucidation of a novel and pure di(alkyloxy)imidazolium based RTIL and its application as extracting agent in LPME for the isolation of CBD from natural cosmetics. To our knowledge, this is the first scientific work dealing with this kind of topic.

It was demonstrated that CBD could be efficiently extracted from different formulated creams and quantified using HPLC–UV analysis. By a two-step DoE optimization a maximum extraction recovery of 80% could be achieved. A validation, including selectivity, linearity, stability, accuracy

and precision of the optimized method was performed and showed satisfying results. A high selectivity in comparison to common extraction procedures was demonstrated by comparing the extraction of pure MeOH with RTIL assisted LPME. In the final step of this study, the optimized and validated method was applied to various cosmetics, including four ointments and four creams from different manufacturers. The CBD concentration could be determined in five out of eight products, which exhibited concentrations ranging from 0.87 to 4.61 mg $g^{-1}$.

Based on the "CBD-boom" during recent years, many CBD cosmetics are now available on the free market. Counterfeit products and products with false declarations will gradually follow this trend and represent a huge problem. It is of utmost importance to control ingredient concentrations, in order to guarantee high qualities. During this study it was observed, that indicated and measured CBD concentrations are not identical, and some products do not even contain CBD (even though it was promoted as CBD product). This fact clearly demonstrates the need of precise and efficient methods for the isolation and detection of CBD from cosmetics.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2297-8739/7/3/45/s1, Figure S1: NMR spectrum of [HEtIMO] in $CDCl_3$, Figure S2: NMR spectrum of [BuEtIMO] in DMSO, Figure S3: NMR spectrum of [BuEtETIMO][$Tf_2N$] in DMSO, Figure S4: Pareto chart of the screening design for categorical parameters, Table S1: Plackett–Burman table from the screening design for categorical parameters, Table S2: Complete ingredients lists of the four salves with an oil-in-water emulsion (O) and the four creams with a water-in-oil emulsion (W) acquired in local shops and Austrian web shops, Table S3: ANOVA results of the full central composite design for numerical parameters for linear and quadratic models, Table S4: Parameters for the determination of LOD and LOQ.

**Author Contributions:** Conceptualization, S.H., M.H., and M.R.; methodology, S.H. and M.H.; validation, S.H.; formal analysis, S.H. and K.F.; investigation, S.H., M.H., K.F., and K.E.; resources, M.P. and G.K.B.; writing—original draft preparation, S.H.; writing—review and editing, M.H., M.P., G.K.B., and M.R.; visualization, S.H.; supervision, G.K.B. and M.K.; All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** We would like to thank the research group of assoc. Christoph Kreutz (Institute of Organic Chemistry, University of Innsbruck) for sharing its expertise in NMR spectroscopy.

**Conflicts of Interest:** The authors declare no conflict of interest.

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