

## Evaluation of measurement uncertainty for purity of a monoterpene acid by small-scale coulometry

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**Abstract:** Purity of the perylic acid (HPE) which is a monoterpene acid from natural product (NP) with anti-inflammatory and anticancer properties was analyzed by small-scale coulometry (SSC), due to the low availability of HPE on the pharmaceutical market and its high cost. This work aims to present the evaluation of the measurements uncertainty from the purity of HPE by using SSC. Coulometric mean of purity obtained from 5 replicates resulted in  $94.23\% \pm 0.88\%$  ( $k = 2.06$ , for an approximately 95% confidence level). These studies aim in the future to develop the production of certified reference materials from NPs.

**Keywords:** Uncertainty, small-scale coulometry, perylic acid

### 1. INTRODUCTION

In the quality control of medicines, the pharmaceutical industry uses chromatographic techniques. However, such techniques require a reference material, to be able to quantify it in the plant, which has become a bottleneck in the development of herbal medicines in pharmaceutical market.

However, the markers of the great majority of medicinal plants originate from their secondary metabolism. These substances are synthesized frequently in small quantities.

Brazil is one of the largest producers of oranges in the world and is the largest exporter of orange juice, with about 17.5 million tons produced in the 2016/2017 harvest, according to data from the

Ministry of Agriculture, Fisheries and Food. Supply (Mapa). Limonene is a by-product of the orange juice industry. The annual production of this monoterpene is estimated at 50,000 tons, and its price is around US \$1–2/kg. It is easily available as waste from the citrus juice industry and is obtained from a renewable source, industries such as pharmaceuticals, chemicals and food have shown great interest in the oxygenated derivatives of limonene, such as HPE.

One of the great obstacles of the natural products (NPs) is to obtain a reference material (RM) compatible with the requirements of the product, according to the new ordinance specific for phytomedicines (RDC 26/14), and considering the high cost of the molecule, the development of a CRM or a RM, will lead to a huge reduction of

analysis costs for the molecule studied.

HPE has a low availability, so it is very expensive. Its structural formula is shown in figure 1.

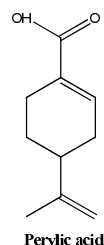


Figure 1. Perylic acid [1].

Certified reference materials (CRMs) are the most important standards for disseminating measurement units (metrological traceability) to users. The CRM specified property values require metrological traceability with associated measurement uncertainty. These studies aim in the future to develop the production of CRMs from NPs.

An electroanalytical technique explained by Faraday's law, applied in the determination of the amount of substance (mol) of pure chemical compounds or their respective solutions and considered primary, since besides its operation is described and understood in terms of SI units, is a constant current Coulometry, that is widely used to determine the purity of RM and has high accuracy.

Microscale chemistry when used requires less reagent consumption and less generation of chemical residues, avoiding environmental pollution. The application of the miniaturization of the coulometric technique is due to the need to use less sample, guaranteeing reliability to the chemical measurements, which, as a primary method, provides measurement results with high precision and accuracy, which are crucial for the development of an CRM of secondary metabolism plant [3]. The traceability and

measurement uncertainty indicate that the key role of the reliability of chemical measurements is the comparability of results from different times and locations. The comparability of results is obtained mainly by evaluation of the measurement uncertainty.

### 1.1. Objective

This work aims to present the measurement uncertainty for purity of a monoterpenic acid determined by SSC.

## 2. EXPERIMENTAL

### 2.1 Materials

All analytical reagent-grade chemicals were used in the development of this work. All the solutions were prepared using deionized water from Elga with an electrolytic conductivity higher than  $0.054 \mu\text{S cm}^{-1}$ . The HPE was purchased from Sigma Aldrich and was certified by the manufacturer to have a 95% purity. Before use, the HPE was dried in an oven at  $80 \text{ }^\circ\text{C}$  for 3 h.

The coulometer used was a high-precision coulometry unit from Applied Precision Ltd. It was assembled with a constant current source (model 8011A) containing a high-precision timer with a precision of  $1 \mu\text{s}$  and an indication unit (model 7011A). A gas system for a high-purity argon supply was used for deoxygenation. Parameters of optimization from SSC can be seen at table 1:

Table 1: Optimization from SSC

Parameters	Optimization from ssc	
	Before	After
Coulometric cell (CC) temperature	$25 \text{ }^\circ\text{C}$	$35 \text{ }^\circ\text{C}$
Medium for sample solubilization	Solvent mixtures <sup>a</sup>	MEtOH <sup>b</sup>
Type of sample transfer to cc	Glass basket	Vacuum
Sample rinsing	KCl 1,0 M	MEtOH
Drying sample before to be used	$70 \text{ }^\circ\text{C}$ , 3 h	$80 \text{ }^\circ\text{C}$ , 3 h
Sample mass reduction scale	50x	100x
Reduction of supporting electrolyte scale	10x	10x

<sup>a</sup>DMSO; ethanol; acetonitrile  
<sup>b</sup>Methanol

The technique used was constant-current

coulometry carried out manually. An Excel spreadsheet was used to perform all the calculations. The sample of HPe was dried at 80 °C for 3 h and cooled in a desiccator over silica gel pellets. Platinum foil (99.99%) with a thickness of 0.25 mm and an area of 20 mm<sup>2</sup> was the generating cathode; a spiral made of silver wire with a purity of 99.99%, from Heraeus Vectra, with dimensions of 50 × 55 × 5 mm was the generating anode. The supporting electrolyte consisted of 25 mL of a 1.0 mol dm<sup>-1</sup> KCl solution. Approximately 10 mL of a similar KCl solution, but with a slightly higher concentration, was introduced in the anode compartment. For the initial titration (endpoint detection at pH ≈ 6.9), approximately 20 μL of 0.01 mol kg<sup>-1</sup> HCl solution (nominal value) was added before a 1.0 mA current was passed during a specific time, ending some time after the endpoint was detected. Each HPe sample of approximately 3.0 mg used in the main titration was weighed in a microbalance to which a buoyancy correction was applied to the weighed mass. In the main titration, a 20 mA current was passed until 80% of the sample was titrated. For the final titration (endpoint detection at pH ≈ 6.9), a 1.0 mA current was passed similarly to the initial titration. The coulometric cell was maintained at 35 °C during the titration. High-purity argon gas (99.999%) was bubbled on both the cathodic and anodic compartments for deaeration or maintained above the cathodic compartment during the main titration. In all the titrations, the supporting electrolyte was stirred with a magnetic stirrer. The Faraday constant, equivalent to 96,485.3289 C mol<sup>-1</sup> and the density of the HPe of 1,007 kg m<sup>-3</sup> were considered in calculations, as was the molar mass for HPe of 166.217 g mol<sup>-1</sup>. The endpoint was calculated using the OriginPro8.5.1 program, considering the secondary derivate of the points of the coulometric titration curve.

The uncertainty measurement evaluation was

carried out according to the specific literature from uncertainty. The evaluation of the measurement uncertainty was performed with consideration of the physical sources of uncertainty, such as the input quantities of the measurand  $\nu$  (amount of substance) according to equation 1 and some important chemical sources for the coulometric titration measurement: endpoint detection, molar mass, current efficiency, diffusion losses and aerosol losses.

$$\nu = \frac{I_1 \cdot t_1 + I_2 \cdot t_2 + I_3 \cdot t_3}{z \cdot F \cdot m \cdot B} \quad (1)$$

where  $\nu$  is the amount of substance;  $I_1$  is the current in A passed in the initial titration;  $t_1$  is the time in s for the initial titration;  $I_2$  is the current in A passed in the main titration;  $t_2$  is the time in s for the main titration;  $I_3$  is the current in A passed in the final titration;  $t_3$  is the time in s for the final titration;  $z$  is the charge of the electrons for the electrochemical reaction;  $F$  is the Faraday constant;  $m$  is the mass sample and  $B$  is the buoyancy correction for the mass sample.

For the evaluation of the uncertainty measurement of the purity in terms of mass fraction,  $w$  (%), it is necessary to consider the molar mass  $M$  of the sample, according to equation. 2.

$$w(\%) = \nu \cdot M \quad (2)$$

### 3. RESULTS

The purity of HPe was determined after the optimization of the methodology of drying and mass sampling by coulometric titration. Table 2 presents the measurement results obtained in 5 different replicates.

Table 2: Coulometric titration

Coulometric titration			
n <sup>o</sup>	m (g)	Amount content (mol kg <sup>-1</sup> )	Mass fraction (%)
1	0.0034271	5.6303	93.586
2	0.0038015	5.6357	93.675
3	0.0036845	5.6752	94.331
4	0.0035127	5.6897	94.572
5	0.0031659	5.7151	94.994
Average (%)		<b>5.6692</b>	<b>94.232</b>
Standard deviation (%)		<b>0.036</b>	<b>0.6</b>
RSD (%)		<b>0.016</b>	<b>0.6</b>

### 3.1 Measurement uncertainty evaluation

For comparability of the measurement results at any time and space it is necessary to express the measurement result followed by its uncertainty. Evaluation of the measurement uncertainty is calculated considering international guides [2,4], which is specifically for chemical analysis. An uncertainty budget of the coulometric titration measurement for HPe in an evaluation considering the relative uncertainty approach is shown in table 3.

Table 3: Uncertainty budget

Source	Value		Standard uncertainty		$c_i \cdot u_i (\nu_{\text{Hpe}})^{-1}$
	Value	Unity	Standard uncertainty	Unity	
Repeatability	5.6691954	mol kg <sup>-1</sup>	$1.61 \times 10^{-2}$	mol kg <sup>-1</sup>	0.28 %
Weighing	0.000003798	kg	$6.5 \times 10^{-10}$	kg	0.02 %
Buoyancy correction	1.00102	l	$9.0 \times 10^{-7}$	l	0.00 %
Time	275.04	s	$5.0 \times 10^{-7}$	s	0.00 %
Current	0.02	A	$9.1 \times 10^{-10}$	A	0.00 %
Faraday constant	96,485.3289	C mol <sup>-1</sup>	$5.9 \times 10^{-4}$	C mol <sup>-1</sup>	0.00 %
End-point detection	-		$5.1 \times 10^{-4}$	mol kg <sup>-1</sup>	0.01 %
Difusion losses	-		$3.0 \times 10^{-3}$	mol kg <sup>-1</sup>	0.05 %
Aerosol losses	-		$1.5 \times 10^{-3}$	mol kg <sup>-1</sup>	0.03 %
Current efficiency	-		$1.5 \times 10^{-2}$	mol kg <sup>-1</sup>	0.27 %
$u_A$	0.28%				
$u_B$	0.28%				
$u_C$	0.40%				

The main sources of uncertainty as can be seen by the Table 3 were the repeatability of the method and the current efficiency. As the method was carried out manually, the precision of the measurements was below than it was expected. On the other hand, the current efficiency was affected, which means that some charge was lost possibly it was used by others secondary electrochemical reactions. The uncertainty budget is also important as a way to indicate the next steps to be followed in order to increase the method performance.

### 4. CONCLUSION

The result of the coulometric titration on a small scale was  $94.2\% \pm 0.9\%$  ( $k= 2.06$ , for an approximately 95% confidence level, according to the specific literature from uncertainty). The purity was also good considering that the sample

was added to the glass coulometric cell without any loss and with a vacuum, although the temperature used in the coulometric cell was 35 °C. The miniaturization of the CC was fundamental for characterizing such substances due to the low availability found in the market, in addition to their high cost. SCC titration results shall be further compared with NMRq results to validate the purity obtained. These studies aim in the future to produce CRMs of NPs. The future development and use of CRM phytomedicines will contribute to the metrological reliability and traceability of the results of gas chromatographic analysis and other analytical measurements used by chemical laboratories for several other NPs that have a similar matrix to those found in the phytotherapeutic one studied here.

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