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Evaluation of the full evaporation technique for quantitative analysis of high boiling compounds with high affinity for apolar matrices☆

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ABSTRACT

In order to reduce inaccuracies due to possible matrix effects in conventional static headspace-gas chromatography (sHS-GC), it is standard practice to match the composition of calibration standards towards the composition of the sample to be analysed by adding blank matrix. However, the latter is not always available and in that case the full evaporation technique (FET) could be a solution. With FET a small sample volume is introduced in a HS vial and compounds of interest are completely evaporated. Hence no equilibrium between the condensed phase and vapour phase exists. Without the existence of an equilibrium, matrix effects are less likely to occur. Another issue often encountered with sHS-sampling is that low vapour pressure compounds with a high affinity for the dilution medium show a limited sensitivity. FET has proven to be an appropriate solution to address this problem too.

In this work, the applicability of FET for the quantitative analysis of high boiling compounds in different complex apolar matrices is examined. Data show that FET is an excellent tool to overcome matrix effects often encountered with conventional sHS analysis. The tested method shows excellent accuracy with recovery values around 100% as well as repeatability with RSD values around 1% for the quantification of high boiling compounds (bp > 200 °C) such as camphor, menthol, methyl salicylate and ethyl salicylate in various matrices. LOQ values were found to be around 0.3 μg per vial. Following validation of the technique, several topical pharmaceutical formulations like ThermoCream®, Reflexspray®, Vicks Vaporub® and Radosalil® were examined. For the latter, a comparison has been made with a sHS-method described in literature.

1. Introduction

Static headspace (sHS) sampling is widely used for the quantitative analysis of volatile compounds in a variety of matrices due to its simplicity and cleanliness of introducing volatiles of interest into a gas chromatograph. A disadvantage of this technique is the possibility of matrix effects causing signal differences between the sample and calibration standards [1]. Matrix effects are any form of interaction in the condensed phase that influences the equilibrium between the condensed and vapour phase in a HS vial. A way to solve this problem is to add blank matrix to calibration standards in order to compensate for the matrix. However, this is not always possible as blank matrix is not always available. So, quantification of volatiles can be inaccurate because matrix effects can influence the established equilibrium between the condensed and vapour phase in a HS vial and thus can give rise to different response factors for a certain compound in different matrices. Possible methods to address this problem are multiple headspace extraction (MHE), standard addition method (SAM) and full evaporation technique (FET). However, MHE can be very time consuming and SAM can only be performed when enough sample is available. In contrast to these techniques, FET only uses very small amounts of sample and does not take more time to perform than a conventional sHS-GC method. With FET, all volatile compounds of interest are transferred completely to the vapour phase and the matrix no longer influences the equilibrium between vapour and condensed phase. FET has proven to be a useful technique to avoid matrix effects during analysis of both solid and liquid samples [2–7]. FET has also proven to be useful for the analysis of high boiling solvents (low vapour pressure) with high affinity for water (high *K* value) in an aqueous matrix. Using conventional sHS-sampling, this determination is difficult since the sensitivity for these high boiling compounds is limited [8]. The same issue regarding sensitivity could arise when high boiling compounds have high affinity for apolar matrices and/or dilution media.

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The aim of this work was to investigate the applicability of FET on the quantification of high boiling compounds (bp > 200 °C) with high affinity for different apolar matrices. The quantitative analysis of camphor (C), DL-menthol (M), methyl salicylate (MeS) and ethyl salicylate (EtS) in different pharmaceutical products was taken as an example. These compounds are usually analysed with either sHS-sampling or direct injection [9–11]. The drawbacks of sHS are mentioned above while direct injection suffers from clogging and sample adsorption in the injector. Aspects like linearity, repeatability and recovery were evaluated. Calibration curves obtained with matrix-matched standards were compared with those obtained with solvent-based standards to check for the absence of matrix effects. In order to evaluate the advantage of FET over sHS for the analysis of apolar high boiling compounds in an apolar matrix, a typical sample containing the aforementioned compounds was analysed with both techniques.

2. Theory

With conventional sHS-GC, an equilibrium exists in a sealed HS vial containing a condensed and a vapour phase, both with a particular volume:

$$V_v = V_g + V_l \quad (1)$$

where V_g is the volume of the vapour phase, V_l the volume of the liquid phase (condensed phase) and V_v the total volume (volume of the vial). If a sample with volume V_0 containing a concentration C_0 is transferred in a vial, the sum of the absolute amounts in the vapour and condensed phase after equilibration equals the total amount of sample before equilibration:

$$C_0 \times V_0 = C_l \times V_l + C_g \times V_g \quad (2)$$

where C_l is the concentration of the analyte in the liquid phase and C_g the concentration in the gas phase. With FET, only a very small amount of sample is transferred into the vial and sufficiently heated. As a result, V_l will approach zero due to near complete evaporation. Consequently Eq. (2) becomes:

$$C_0 \times V_0 = C_g \times V_g \quad (3)$$

so that the amount of the compound of interest in the gas phase is directly determined by the sample amount in the HS vial. The approximate maximum volume of solvent and absolute amount of compounds that can be introduced in a HS vial in order to meet the criterion for FET can be calculated by combining Antoine's equation and the Ideal Gas law [8]. When the maximum amount of a certain compound is exceeded, condensation will occur and the requirements for FET will not be met as the system is in sHS-mode, and hence, possible matrix effects can occur. When performing FET-analysis, the compound that has the highest abundance in the vial is the solvent. It has to be ensured that the pressure at equilibrium in the vial does not exceed the pressure applied on the vial during injection. The latter pressure is determined by the chromatographic conditions. During heating of the vial, the air that is present in the vial will also exert a certain pressure depending on the temperature (thermal expansion). When the applied injection pressure is not high enough to counteract the equilibration pressure, an amount of gas phase will flow into the injection system when the needle punctures the vial. This will result in loss of sample with pressure loop systems or pre-injection with balanced pressure systems. Therefore, in practice the maximum amount of solvent that can be introduced in a vial will be lower than the calculated maximum amount that can be evaporated.

3. Experimental

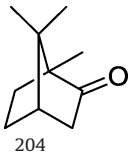
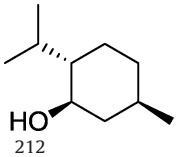
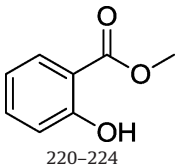
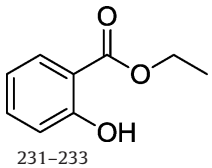
3.1. Reagents and samples

C, M, MeS, EtS, (structure, boiling points and Antoine's constants are given in Table 1) and Radosalil® stick were kindly donated by Will Pharma (Wavre, Belgium). Salicylic acid, paraffin, petroleum jelly, cetiol, capsaicin oleoresin (containing 8% of capsaicin) and Lanette SX for the preparation of blank Radosalil® matrix were obtained from Will Pharma as well. ThermoCream® and its blank matrix were obtained from Sterop (Brussels, Belgium). Reflexspray® and Vicks Vaporub® were obtained from a local pharmacy. 1-Octanol (98%) was obtained from Janssen Chimica (Geel, Belgium), *o*-xylene (99%) from VWR International (Heverlee, Belgium), DMF from Fischer Chemical (Loughborough, United Kingdom). The boiling points and Antoine's coefficients of *o*-xylene and DMF are given in Table 1 as well. *n*-Dodecane was purchased from Sigma-Aldrich (Diegem, Belgium). The composition of each matrix is given in Table 2.

3.2. Chromatographic system and sampling conditions for the sHS-method

FET was compared with a sHS-method described in literature [9] used for the quantification of C, M, MeS and EtS in Radosalil®, a typical petroleum jelly/paraffin based matrix for which these compounds have a high affinity. The method was slightly adapted by using *n*-dodecane as solvent as the prescribed liquid paraffin does not allow to work with volumetric flasks.

Table 1
Chemical structure, boiling points and Antoine's constants of the investigated compounds and used solvents.

Compounds	C	M	MeS	EtS
Chemical structure				
Boiling point (°C)	204	212	220–224	231–233
**Antoine's constant A	3.30967	5.383470	4.02455	*
**Antoine's constant B	1096.291	2405.946	1585.874	*
**Antoine's constant C	−148.579	−37.853	−99.173	*
Solvent	*o*-Xylene		DMF	
Boiling point (°C)	144.4		153	
**Antoine's constant A	4.12928		3.93068	
**Antoine's constant B	1478.244		1337.716	
**Antoine's constant C	−59.076		−83.648	

* Antoine's constants unknown.
** From NIST data.