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Use of microextraction by packed sorbents and gas chromatography-mass spectrometry for the determination of polyamines and related compounds in urine

Ana María Casas Ferreira[a,*], Bernardo Moreno Cordero[a], Ángel Pedro Crisolino Pozas[b], José Luis Pérez Pavón[a]

[a] Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias Químicas, Universidad de Salamanca, 37008 Salamanca, Spain
[b] Servicio de Medicina Interna, Hospital Virgen de la Vega, Complejo Asistencial Universitario de Salamanca, 37007 Salamanca, Spain

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ABSTRACT

A novel methodology for the determination of ornithine, putrescine, cadaverine, spermidine and gamma-amino butyric acid in urine samples has been developed. The method uses *in situ* aqueous derivatization followed by automated microextraction by packed sorbent coupled to a gas chromatography–mass spectrometry system equipped with a programmed temperature vaporizer. This instrumental configuration minimizes sample manipulation due to from the mixing of the reagents, the process is completely automated. The analytes were derivatized using ethyl chloroformate as derivatization reagent. The reaction occurred in aqueous medium and was carried out in 1 min in the vial of an autosampler used to perform microextraction by packed sorbent. The parameters affecting derivatization, extraction and separation were optimized in order to obtain maximum sensitivity. Calibration curves were obtained for five calibration levels in three different matrices. All the calibration models displayed good linearity, with R^2 values higher than 0.95. The validity of the models was checked using ANOVA, and it was observed that they did not exhibit any lack of fit. Repeatability and reproducibility was evaluated, with values below 15% in both cases. LOD and LOQ values were found to be in the low μg/L level. Influence of the matrix was confirmed, thus quantification was performed using the standard additions method and normalization to IS. The method developed was applied to the analysis of these compounds in urine samples from healthy individuals and cancer diagnosed patients (Internal Medicine Unit of the Virgen de la Vega Hospital, Salamanca, Spain). Significant differences (Mann-Whitney U test) were observed for putrescine and ornithine concentrations.

1. Introduction

Since microextraction by packed sorbents (MEPS) emerged in 2004 [1], it has been accepted as a powerful sample preparation approach to a range of analytical and bioanalytical challenges [2–4]. MEPS is based on similar principles as SPE, but presents some significant differences: the MEPS packing material (~2 mg) is directly incorporated within the sample syringe (not in a separate column), can handle small volumes of sample (10 to up to 5000 μL), can be connected on-line to different chromatographic techniques, including GC [1,5–7] or LC [8–10], and can be used more than 100 times for plasma or urine samples. In addition, since MEPS is an on-line device using the same syringe, all necessary steps including sample processing, extraction and injection steps can be automated [2].

Polyamines are ubiquitous aliphatic amines with low molecular weight, which play a critical role in many mammalian processes, such as DNA synthesis and stability, transcription and ion channel regulation, and protein phosphorylation [11]. Polyamine biosynthesis in mammalian cells begins with the production of putrescine from L-ornithine, an amino acid that is not found in proteins and that is produced as part of the urea cycle. Putrescine is the precursor used in the spermidine and spermine biosynthesis, while cadaverine is produced by the decarboxylation of lysine [12]. Although urea-cycle enzymes are expressed primarily in the liver and intestine, polyamines are synthesised in all tissues. Polyamines are also obtained from the diet and other sources such as intestinal

* Corresponding author.
E-mail address: anacasas@usal.es (A.M. Casas Ferreira).

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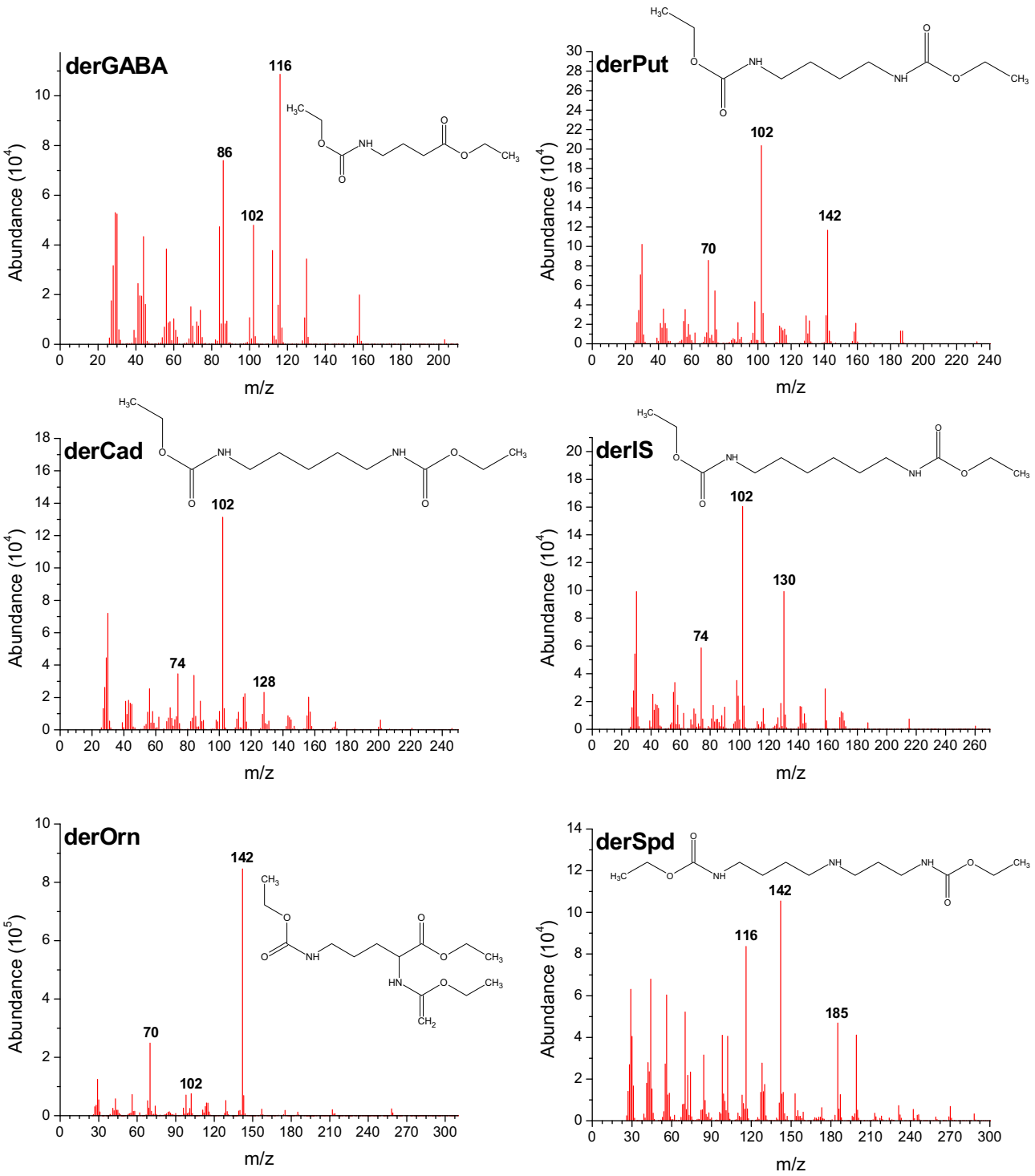

Fig. 1. Electron impact (70 eV) mass spectra of the derivatized compounds.

bacteria. These exogenous polyamines are transported into cells from extracellular space [13].

Polyamines have long been associated with cell proliferation and tissue growth, and are known to be involved in the development of several tissue types. The association of increased polyamine synthesis with cell growth and cancer was first reported in the late 1960s [13,14]. Russell and Snyder reported high levels of ornithine decarboxylase, the enzyme required for the first stage in polyamine synthesis, in regenerating rat liver and in several human cancers [14]. Since then, a number of studies have indicated higher concentrations of polyamines in cancer patients compared to healthy subjects [15]. These significantly increased levels have been associated with increased secretions from the proliferating cells themselves or with release from dead cells, as a consequence of the active replacement of cell growing tissues [15]. However, their use as cancer biomarkers remains controversial. Variations in polyamine levels have also been reported in diseases such as cystic fibrosis, muscular dystrophy or rheumatoid arthritis [15].

Liquid chromatography (LC) methods have primarily been used for the determination of these compounds in biological