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A rapid LC–MS method for determination of plasma anion profiles of acidotic patients

William McKinnon [a], Gwyn A. Lord [b], Lui G. Forni [c], Jean-Marie R. Peron [d], Philip J. Hilton [a,*]

[a] *Renal Research Laboratory, Department Medicine, 4th Floor, North Wing, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK*
[b] *MRC Bioanalytical Science Group, School of Biological and Chemical Sciences, Birkbeck, University of London, Malet Street, London WC1 7HX, UK*
[c] *Department of Critical Care Medicine, Worthing Hospital, Worthing, West Sussex BN11 2DH/Brighton & Sussex Medical School, Sussex, UK*
[d] *School of Pharmacy and Chemistry, Kingston University, Kingston-upon-Thames, Surrey KT1 2EE, UK*

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Abstract

In metabolic acidosis, the concentrations of anions associated with intermediary metabolism are increased and can make a significant contribution to the observed acidosis. Here we describe a method for the rapid determination of the plasma ultrafiltrate profile of these anions using liquid chromatography coupled to electrospray ionisation mass spectrometry (LC/ESI-MS). The ultrafiltrate from patients with acidosis resulting from various causes were examined and the results compared to control values. Using the LC/ESI-MS method described, a unique plasma ultrafiltrate anion profile was obtained for each of the groups studied that provides rapid diagnosis of the type of underlying acidosis.

Keywords: Acidosis; Patients; Plasma; Ultrafiltrate; Krebs cycle; Anion gap; Intermediatry metabolism; Liquid chromatography; Mass spectrometry

1. Introduction

We have previously shown that the levels of anions normally associated with intermediary metabolism are altered in certain acidotic conditions and can contribute significantly to the development of the observed anion gap in these patients [1]. The absolute concentrations of these anions can be measured in a quantitative manner using enzyme assay. However, this method is slow and it can take several hours to determine the concentration of each individual anion. Gas chromatography or capillary electrophoresis coupled to a mass spectrometer have previously been used to examine anions in biological matrices but gas chromatography requires complex and time consuming chemical derivatisation of the anion(s) [2]; furthermore, by-products from this derivatisation have been reported to interfere with analysis. Other groups have reported the use of capillary electrophoresis to determine the anions present biological matrices, but this requires methodologically and technically demanding techniques in addition to time consuming extractions and chemical derivatisation [3]. Other authors have reported that if organic acids are to be measured in the serum of critically ill patients the measurement must be performed quickly due to the rapid metabolism of the anions [4].

With the increasing availability of robust liquid chromatographic separation coupled to on-line electrospray ionisation mass spectrometry (LC/ESI-MS) in hospital diagnostic laboratories we have developed a technique to allow rapid and routine determination of the anion profile in the plasma ultrafiltrate of patients. The determination of organic acids by HPLC has the advantage of being rapid, methodologically easier and more economical than many other techniques [5].

Although liquid chromatography of anions from biological matrices has been reported previously [5,6] these techniques have relied upon single wavelength UV detection coupled with the retention time to determine perturbations in the anion profile from a complex chromatogram. On-line LC/ESI-MS analysis, that would allow unequivocal identification of anions present in plasma has previously been regarded as impractical as a consequence of the presence of sulphuric acid in the mobile phase of ion exchange methods. We have developed a method utilising LC/ESI-MS that allows the unequivocal and rapid determination of the profile of relatively low molecular weight anions normally

* Corresponding author. Fax: +44 207 928 4458.
E-mail addresses: g.lord@sbc.bbk.ac.uk (G.A. Lord), lui.forni@wash.nhs.uk (L.G. Forni), jmperon@f2s.com (J.-M.R. Peron), renallab@gmail.com (P.J. Hilton).

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associated with intermediate metabolism from plasma ultrafiltrate and applied the findings to develop a method that allows the type of the underlying acidosis to be rapidly determined.

2. Experimental

This study was approved by the ethics committee of Guy's and St. Thomas' NHS Trust (Ref Number EC03/104). Prior to the sample being obtained, informed consent was obtained from the subject or where this was not possible, their next of kin.

2.1. Materials

All chemicals and solvents were of analytical or HPLC grade and were used without further purification. Unless stated otherwise, all the chemicals and solvents were purchased from Sigma Chemicals Ltd. (Poole, Dorset, UK). Enzymatic determination of the concentration of citrate, succinate, malate, D- and L-lactate acids levels in plasma ultrafiltrate were estimated using commercially available kits (Roche, Glasgow, UK). The levels of isocitrate and α-ketoglutarate were measured using our own enzyme assay utilising isocitrate dehydrogenase and α-ketoglutarate dehydrogenase respectively and their associated co-factors (Sigma Chemicals Ltd., Poole, UK). These assays are described elsewhere [1].

2.2. Instrumentation and chromatograpic conditions

We used an Agilent HPLC system (Agilent 1100) which consisted of a quaternary pump and on-line degasser coupled to a Series 1100 Mass-Spectrometer fitted with electrospray ionisation and operating in 'negative ion' mode (Agilent Technologies UK Ltd., Wokingham, Berkshire, UK). Purification of the anions in the sample was attained by use of an Aminex HPX-87H Ion Exclusion Column (300 mm × 7.8 mm, Bio-Rad, Hemel-Hempstead, Herts, UK). These columns were supplied from the manufacturers with the resin bathed in 0.008 M sulphuric acid. Since the presence of sulphuric acid in the ESI-MS suppresses analyte ionisation, it was removed prior to use by washing the column for approximately 150 h with mobile phase at a flow rate of 0.8 ml min^{-1}. Preliminary work had previously shown that the sulphuric acid could be replaced by HCl with no degradation of either column resolution or working life. Whilst in use the column was surrounded by a water jacket fed from a water bath maintained at 31 °C. Post-column, the eluent was split so that approximately 8% of the flow entered the electrospray nebuliser, the remainder being diverted to waste. This split was attained by use of a "T" piece and differing resistances to flow induced by the use of tubing of differing internal diameters (id) and length. The eluent was introduced to the "T" piece through PEEK tubing of 0.75 mm id. The arm that split the flow to waste consisted of approximately two metres of PEEK tubing of 0.25 mm id. The arm that carried the flow to the nebuliser consisted of a piece of PEEK tubing 14 cm long of 0.75 mm id.

Prior to entering the MS source the HCl in the stream was partially neutralised by the addition of 10 mM ammonium acetate in a 50:50 (v/v) methanol/water mixture at a flow rate of 0.09 ml h^{-1} through a second "T" piece. The eluent was then introduced to the ESI source of the MS via 8 cm of PEEK tubing of 0.25 mm id (outlined in Fig. 1).

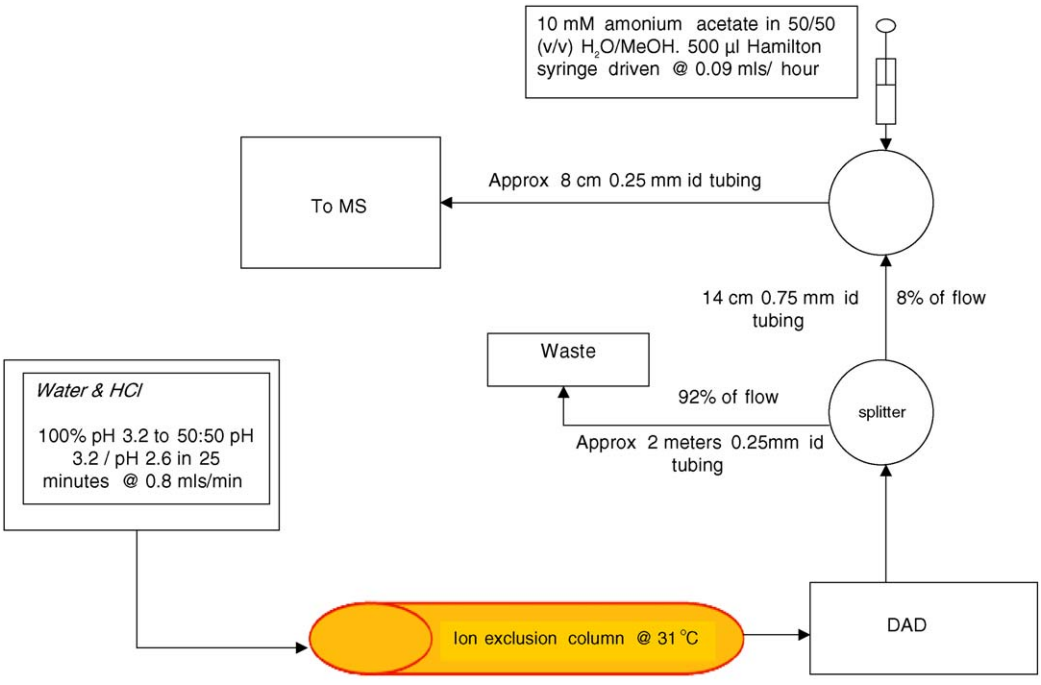

Fig. 1. Schematic layout for the on-line measurement of anions usually associated with intermediate metabolism in human plasma ultrafiltrate using liquid chromatography coupled with negative ion electrospray mass spectroscopy.