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Application of particle-beam mass spectrometry to drugs

An examination of the parameters affecting sensitivity

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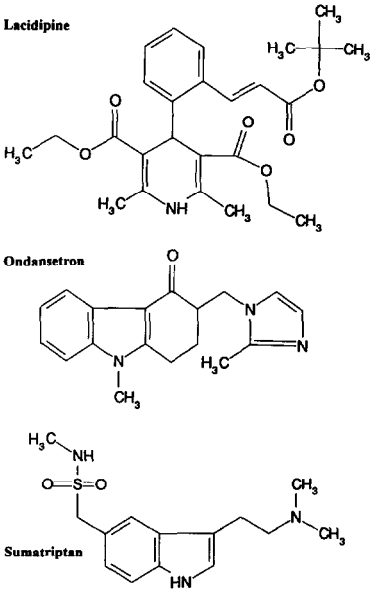
ABSTRACT

The qualitative performance of a particle-beam interface is evaluated by an examination of the effects of altering the pressure of the nebulisation gas, the nebuliser position, the desolvation chamber temperature, the temperature of the nebulisation gas, the temperature of the source and the solvent composition. All these parameters have an impact on performance excepting the nebulisation gas temperature. A summary of the "optimum" settings for routine operation is given.

INTRODUCTION

In recent years several new liquid chromatography–mass spectrometry (LC–MS) interfaces have become commercially available, increasing the range of compounds amenable to analysis by LC–MS; one of these being the particle-beam interface. Several papers have been published dealing with the optimisation of the interface [1] and outlining some of its limitations such as linearity [2], peak broadening [3] and matrix effects [4]. The performance of the interface in this laboratory did not reflect that expected, based upon published data [1], so it was thought prudent to perform an evaluation of the interface to determine what parameters concerned with its operation are crucial to performance.

This paper describes an evaluation of the particle-beam interface using three drug compounds of commercial interest to Glaxo [Lacidipine (anti-hypertensive, M_r 455.56), Ondansetron (anti-emetic, M_r 293.37) and Sumatriptan (anti-migraine, M_r 295.41)]. The parameters examined were the effects of altering the pressure of the nebulisation gas, the nebuliser position, the desolvation chamber temperature, the temperature of the nebulisation gas, the temperature of the source and the solvent composition. The solvent flow-rate is known to be of importance with a recommended rate of 0.4–0.6 ml/min being optimum [1]. In this laboratory flow-rates much in



excess of 0.5 ml/min with high aqueous content have resulted in the formation of liquid droplets inside the desolvation chamber. Therefore all experiments were performed at a flow-rate of 0.5 ml/min.

EXPERIMENTAL

Equipment

The HPLC–MS was performed using an LCC 2252 controller with two 2248 pumps (Pharmacia Biosystems, Milton Keynes, UK) with the flow set to 0.5 ml/min coupled to an HP59980B particle-beam interface and an HP5989A MS-ENGINE quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA) scanning 120–650 amu per 0.6 s. Chemical ionisation spectra were obtained using ammonia reagent gas and an electron energy of 230 eV. The fused silica used in the nebuliser assembly has an I.D. of 0.200 mm. These are available in pre-cut lengths (8.5 cm) (Hewlett-Packard).

Chemicals

Methanol (HPLC grade) was obtained from Rathburn (Walkerburn, UK), acetonitrile (HPLC solvent) and ammonium acetate (FSA laboratory supplies, Loughborough, UK). Distilled water was prepared in-house. Lacidipine, Ondansetron and Sumatriptan were obtained in-house and dissolved in acetonitrile, methanol and methanol–water (50:50) respectively.

Design

The reason for this evaluation was to determine performance trends, and thus identify an "optimum" set of parameters for day-to-day operation of the interface. Therefore the absolute responses obtained for the test compounds were of no interest. This made experimental design much easier.

Each experiment consisted of a series of flow injections (20 μl) of the test compounds with changes made in either the pressure of the nebulisation gas, the nebuliser position, the desolvation chamber temperature, the temperature of the nebulisation gas, or the temperature of the source in conjunction with changes in the solvent composition. Each data point was the average of three injections where the mass spectral response had been determined using the automated quantitation routine. The most intense response was taken as 100% and all others calculated as percentages of that.

Experiments were initially carried out on all three drugs with the solvent compositions methanol–water (25:75), (50:50) and (75:25) and then using only Ondansetron with the solvent compositions acetonitrile–water (25:75), (50:50) and (75:25).

RESULTS

The nebulisation gas pressure has a marked effect on the response obtained with solvent compositions methanol–water (25:75), (50:50) and (75:25) (Fig. 1). A 10-fold increase in response was obtained as the pressure is increased from 30–70 p.s.i. (1 p.s.i. = 6894.76 Pa). With solvent compositions acetonitrile–water (50:50) and (75:25) (Fig. 2) the improvement is less than 2-fold though at acetonitrile–water (25:75) the increase in response is 5-fold. These data clearly

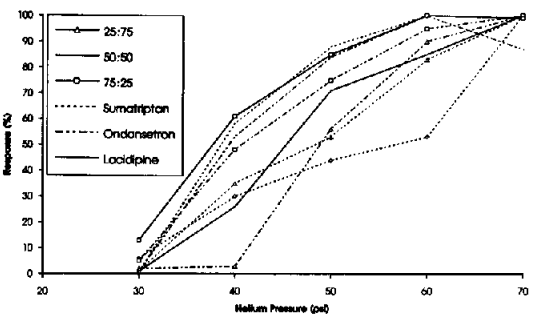


Fig. 1. Effect of helium pressure on response (methanol–water).

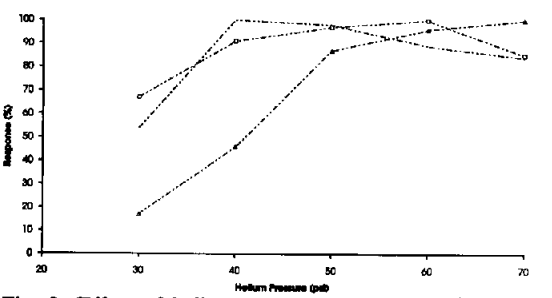


Fig. 2. Effect of helium pressure on response (acetonitrile–water). For symbols see Fig. 1.

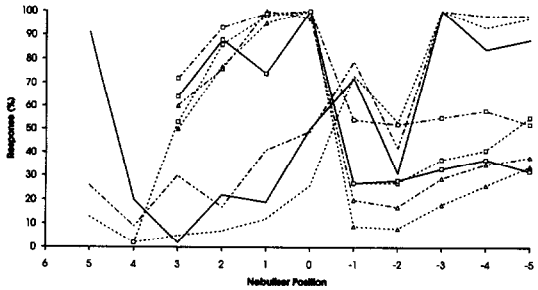


Fig. 3. Effect of nebuliser position at 40 p.s.i. (methanol–water). For symbols see Fig. 1.

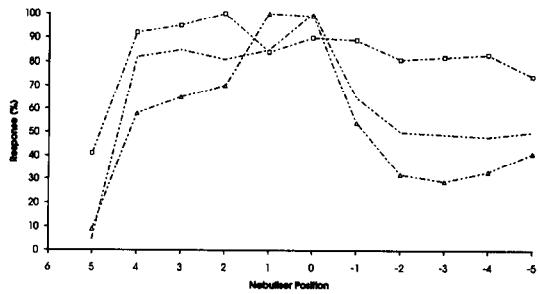


Fig. 6. Effect of nebuliser position on response at 60 p.s.i. (acetonitrile–water). For symbols see Fig. 1.

indicate that the nebulisation gas pressure has a most significant effect on the transmission of analyte molecules through the particle-beam interface.

The position of the fused-silica capillary inside the nebuliser body had a significant impact on the response obtained. It was observed that with a nebulisation gas pressure of 40 p.s.i. (Fig. 3) the position of the nebuliser could afford as much as a 10-fold increase in response. At 60 p.s.i. (Fig. 4) the variations in response were less severe but still significant. A similar response profile was obtained with Ondansetron using acetonitrile (Figs. 5 and 6) instead of methanol. It was observed that with a nebulisation gas pressure of 40 p.s.i. there appeared to be an optimum position when the fused-silica capillary was flush or protruding a little from the nebuliser body at all solvent compositions, except at methanol–water (50:50) when the optimum appeared to occur with the capillary retracted into the nebuliser body.

Increasing the desolvation chamber temperature afforded a variable improvement in response while using either methanol or acetonitrile (Figs. 7 and 8). Though it was quite clear

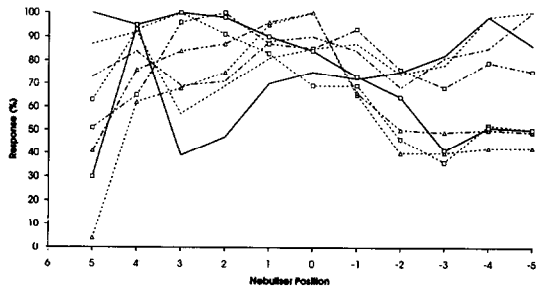


Fig. 4. Effect of nebuliser position at 60 p.s.i. (methanol–water). For symbols see Fig. 1.

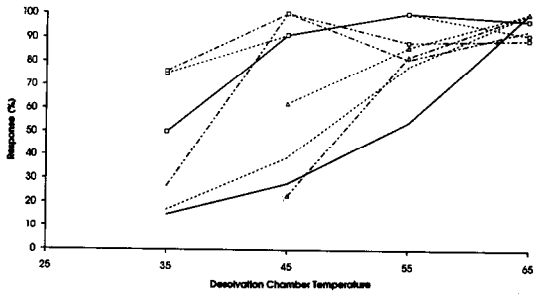


Fig. 7. Effect of desolvation chamber temperature (°C) on response (methanol–water). For symbols see Fig. 1.

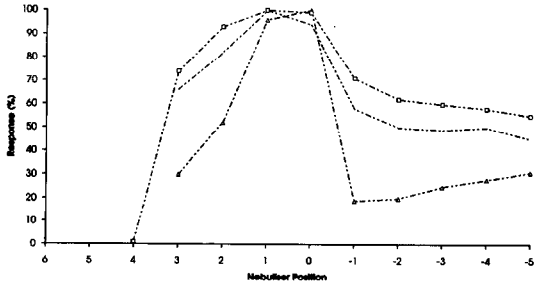


Fig. 5. Effect of nebuliser position on response at 40 p.s.i. (acetonitrile–water). For symbols see Fig. 1.

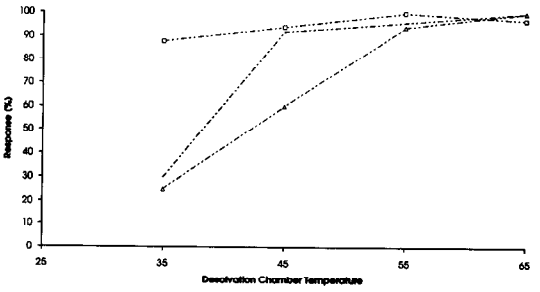


Fig. 8. Effect of desolvation chamber temperature (°C) on response (acetonitrile–water). For symbols see Fig. 1.

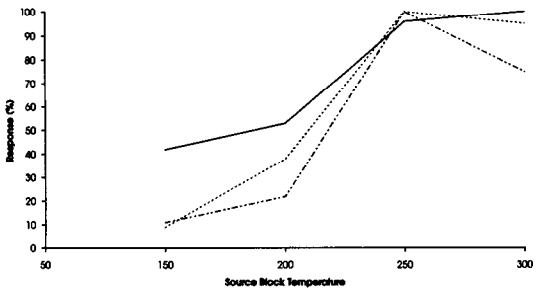


Fig. 9. Effect of source block temperature (°C) on response. Nebuliser gas pressure, 60 p.s.i.; desolvation chamber temperature, 55°C; solvent composition, methanol–water (50:50).

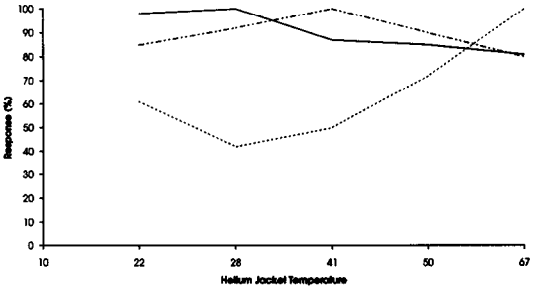


Fig. 11. Effect of heating helium on response at 60 p.s.i. Nebuliser gas pressure, 60 p.s.i.; desolvation chamber temperature, 55°C; solvent composition, methanol–water (50:50). Helium jacket temperature in °C.

that an improvement in response was obtained at higher temperatures. This must reflect both the mass of the analyte and the susceptibility of the various solvent compositions to desolvation.

The remaining experiments were carried out with a nebuliser gas pressure of 60 p.s.i., a desolvation chamber temperature of 55°C and with a solvent composition of methanol–water (50:50).

Changing the source block temperature from 150–300°C (Fig. 9) resulted in as much as a 10-fold increase in response for Sumatriptan and Ondansetron, but only a 2.5-fold increase for Lacidipine. This is likely to reflect the chemical properties of the anlayte as well as the degree of desolvation already achieved during passage through the interface.

Heating the helium nebulisation gas afforded, if anything, a decrease in response with a nebulisation gas pressure of 40 p.s.i. (Fig. 10). There was no apparent trend with a nebulisation gas pressure of 60 p.s.i. (Fig. 11). Possibly the nebulisation gas pressure and the desolvation chamber temperature masked any increase in response. If that were the case the increases in response could only have been very minor.

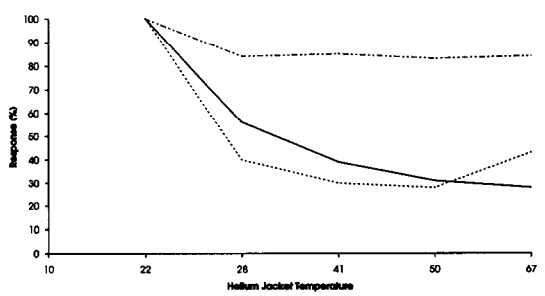


Fig. 10. Effect of heating helium on response at 40 p.s.i. Nebuliser gas pressure, 60 p.s.i.; desolvation chamber temperature, 55°C; solvent composition, methanol–water (50:50). Helium jacket temperature in °C.

Addition of 0.1 *M* ammonium acetate to the solvent system resulted in a 2.5-fold increase in response for both Sumatriptan and Ondansetron with a 1.5-fold increase for Lacidipine. Increasing the concentration of the ammonium acetate did not appear to afford any further increase in response, such "carrier effects" have been previously reported [5]. Lacidipine affords less of an increase probably due to its greater mass than either Ondansetron or Sumatriptan, such that the "carrier effect" is reduced.

CONCLUSION

Examination of the results obtained indicate that the nebulisation gas pressure, nebuliser position, desolvation chamber temperature and source temperature all have a significant impact on sensitivity. It was also observed that the optimum settings at one solvent composition were often close to optimum for most other solvent compositions. Heating the helium nebulisation gas appeared to offer no improvement in sensitivity.

Following this study the particle-beam interface is routinely operated in this laboratory using the following conditions: (i) helium nebulisation gas pressure 60 p.s.i.,(ii) nebuliser position 0–1

(so the silica capillary protrudes a little), (iii) desolvation chamber temperature 55°C, (iv) source block temperature 250°C, (v) 0.2 *M* aqueous ammonium acetate.

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