



The Combined Quantitation of Two Challenging Compounds, Fluticasone and Salmeterol, on a New Tandem Mass Spectrometer

AUTHORS

Edward Brewer²; Min Meng²; Patrick Bennett²; Spencer Carter²; George Scott¹; Robert Horton²; Gerard Dalglish²

¹Ionics Mass Spectrometry Group, Bolton, Canada; ²Tandem Labs, West Trenton, NJ

OVERVIEW

Fluticasone and Salmeterol combinations are used to treat asthma. However because of their differences in polarity and fragility, they are very difficult compounds to assay simultaneously. The assay's sensitivity to high chemical noise, being sticky and requiring an LOQ in the low femtomole level, makes it an excellent test for the performance of a new mass spectrometer. The new mass spectrometer presented, introduces analytes through a heated interface which has an orthogonal entrance and exit, which in combination with low velocity sampling of the gas flow, significantly helps decrease the contamination of the instrument.

EXPERIMENTAL METHODS

The mass analysis was run on an Ionic 3Q Molecular Analyzer (from Ionics Mass Spectrometry Group of Bolton, Ontario) in the positive ion mode with an coaxial electrospray ion source. Fluticasone LC/MRM transition monitored was 501/313 and the Salmeterol transition was 416/380. The Coaxial Electrospray was run at 550°C with a nebulizer pressure of 70 psi and an auxiliary gas flow of 5L/min. The HSID was set at 150°C with a drying gas flow of 1L/min. The sample entrance potential differential between the entrance cap and Q1 is set to zero. The ions enter the mass spectrometer by gas flow dynamics rather than electrostatics.

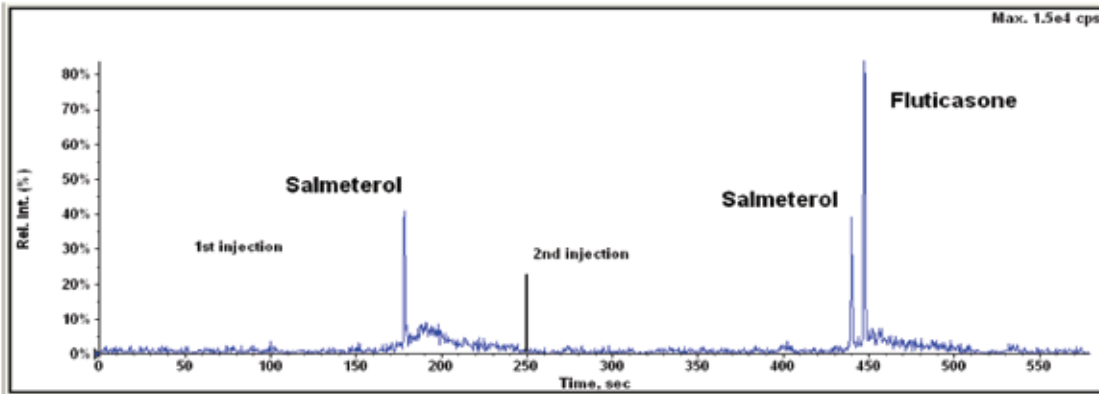
The sample's HPLC separation was accomplished on a Shimadzu Prominence XR UFLC using a C18 betabasic at 0.5 mL/min., with 10 microliters injected. The HPLC separation used 10/45/45 water/acetonitrile/methanol in 10 mM ammonium formate at pH 3.

The methanol, water, and acetonitrile are HPLC grade from Caledon Labs of Georgetown, Ontario. The formic acid, ammonium formate (#516961), Salmeterol Xinafoate (S5068), Fluticasone Propionate (F9428) are from Sigma-Aldrich. The analytical column was a 50 X 2.1 mm 3.5 micron HPLC column from Thermo Fisher Scientific.

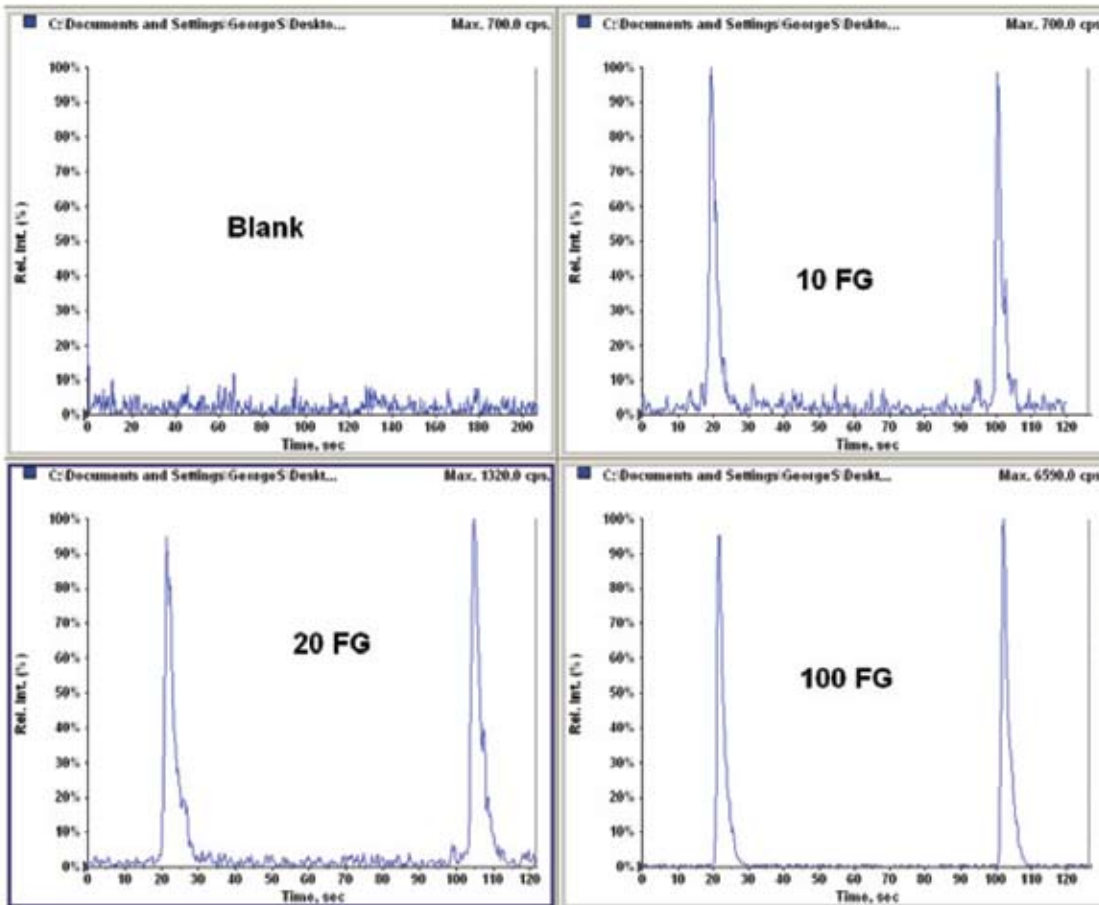


RESULTS

LC MRM TIC of an injection of Salmeterol and a 2nd injection of Salmeterol and Fluticasone



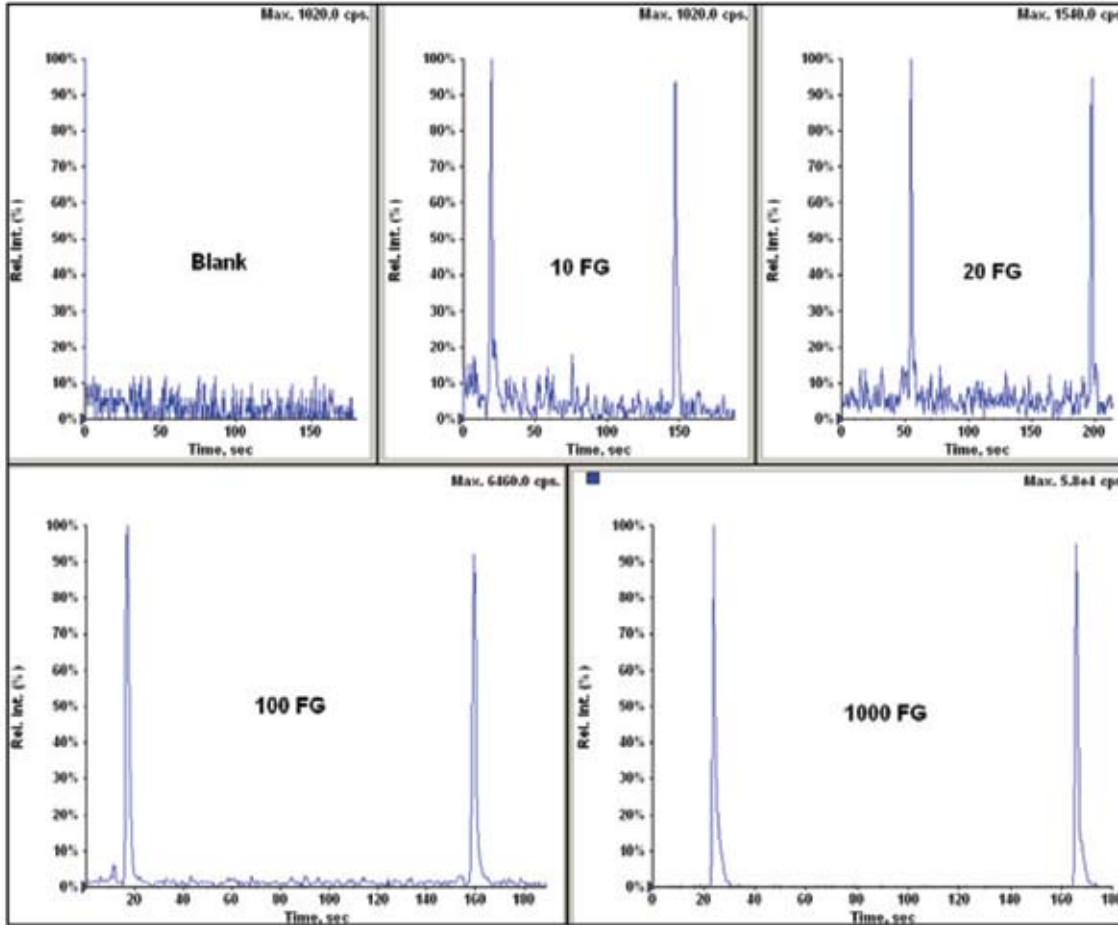
Fluticasone LC MRM 501/313





RESULTS continued

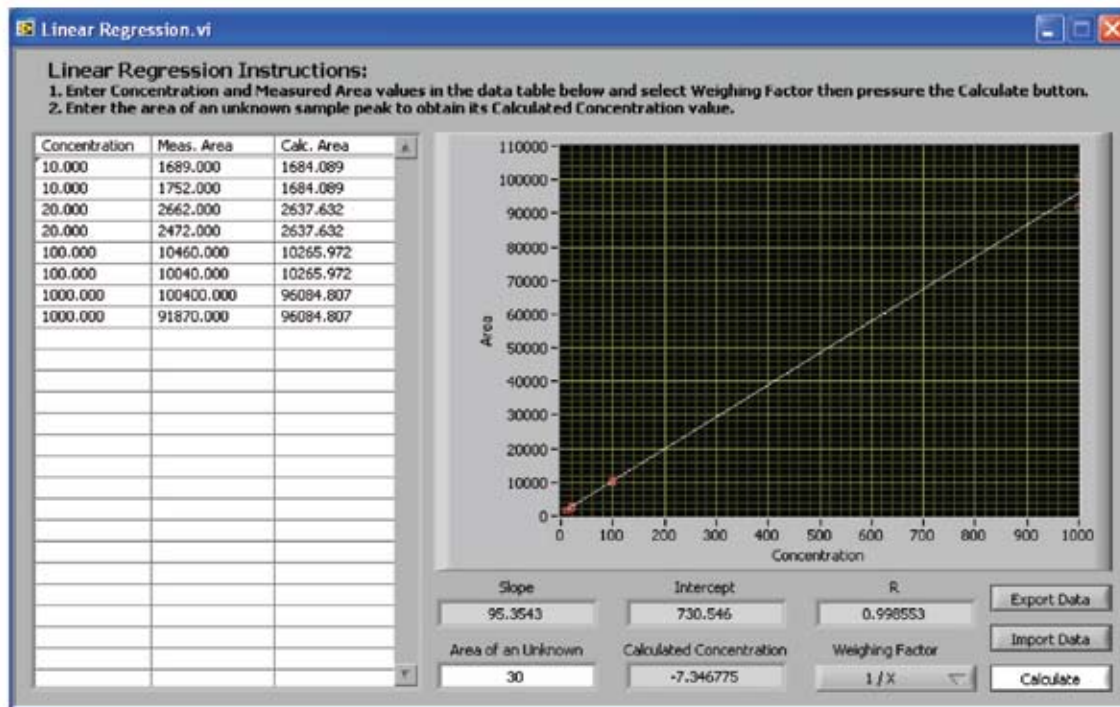
Salmeterol LC MRM 416/380



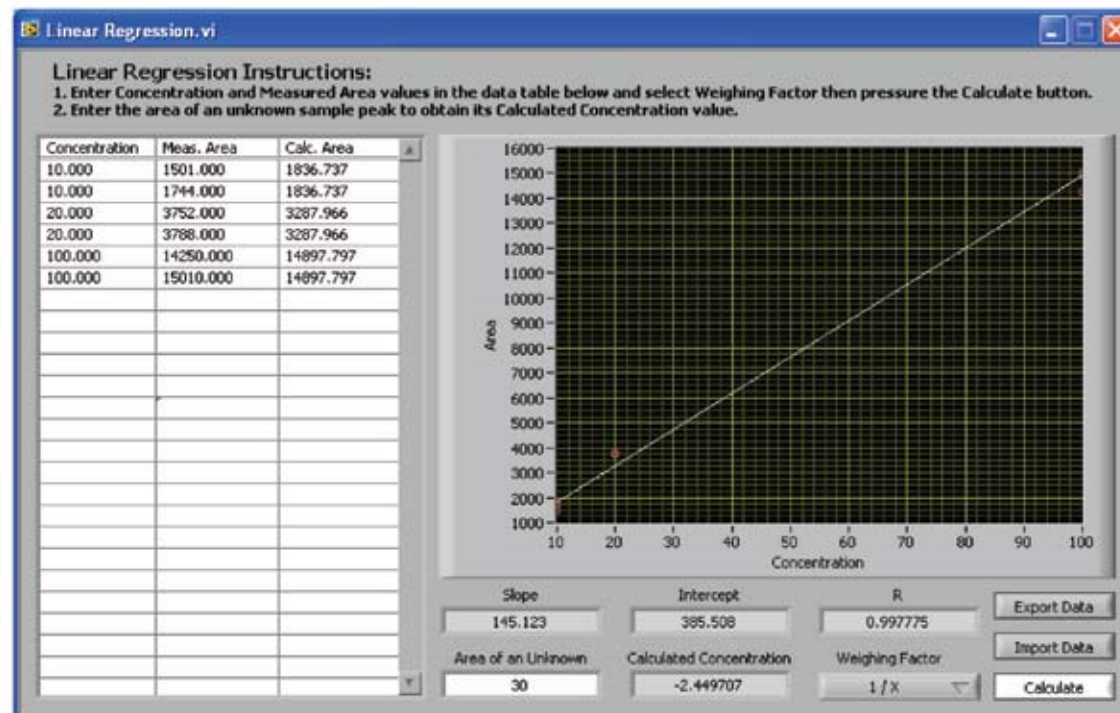


RESULTS continued

Salmeterol LCMRM 416/380 Calibration Curve



Fluticasone LC MRM 501/380 Calibration Curve





CONCLUSION

Optimization and evaluation of the compounds Fluticasone and Salmeterol were performed on the mass spectrometer to determine the best mode of operation. Electrospray in the positive ion mode proved to be the best choice for the analysis of the mixture. The MRM combination of 501/313 and 416/380 in the positive mode were used. The instrument's new interface design and method of transporting ions proved successful in overcoming the difficulties in running the assay and achieving low femtogram sensitivity. Both compounds demonstrated good sensitivity in the low femtogram level with linearity coefficients better than 0.997. These compounds did prove to be a challenge with respect to the HPLC separation and contamination.