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AUTOMATION OF ON-LINE SAMPLE PREPARATION FOR LC APPLICATIONS

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INTRODUCTION

Sample preparation is an essential part of any analytical workflow. In fact, good quality data can only be achieved when counting on a robust and reproducible sample preparation. Nevertheless, sample preparation can be very time consuming, especially for high throughput purposes.

On-line automation of sample preparation can not only guarantee control over the way the sample is prepared through the whole sample batch but can also significantly optimise time and analyst efforts.

This application note describes the automation of sample preparation of three different product samples for high throughput LC applications.

INSTRUMENTATION

The fully automated workflows were developed on a GERSTEL Dual Head MPS system (Figure 1) equipped with the following objects:

- MPS xt Stainless Steel Injection Valves Cheminert
- 2x 3 Solvent reservoirs 100 mL (total of 6 positions)
- Modular wash station
- Tray VT32-10 mL
- GERSTEL multiposition vortexer (mVorx)
- Anatune CF-200 Robotic Centrifuge

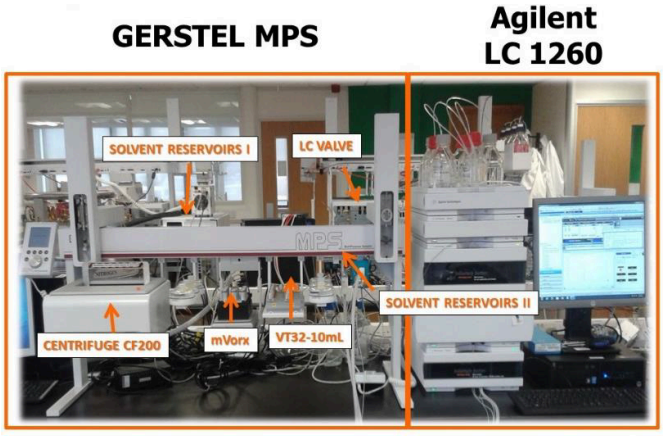

Figure 1: Full solution GERSTEL Dual Head MPS for automated sample preparation for LC applications

METHOD

Optimised Automated Sample Preparation

Tablet: one whole tablet was transferred to a 10 mL glass screw vial. 2 mL of water were added and the sample was then vortexed for 20 minutes. 2 mL of methanol and 1 mL of internal standard solution (benzophenone in methanol 40 mg/mL) were added to the same vial and the sample was further vortexed for 20 minutes. Once agitation was completed, the sample was centrifuged at 4500 rpm for 5 minutes. An aliquot of supernatant solution was diluted to 10 mL with sample solvent and vortexed for 1 min at 3000 rpm to homogenize.

Lozenge: one crushed lozenge was transferred to a 10 mL glass screw vial. 4.5 mL of sample solvent was added and the sample was vortexed for 5 minutes at 3000 rpm. Additional 4.5 mL of sample solvent was added to the sample and the sample was further vortexed for 10 minutes at 3000 rpm. An aliquot of solution was diluted to 10 mL with sample solvent and vortexed for 2 minutes at 3000 rpm to homogenize.

Liquid: approximately 3.5 g of liquid sampler were weighed in a 10 mL glass screw vial. 5 mL of sample solvent was added and the sample was then vortexed for 2 min at 3000 rpm. An aliquot of the obtained solution was diluted to 10 mL with sample solvent and vortexed at 3000 rpm for 2 minutes to homogenize.

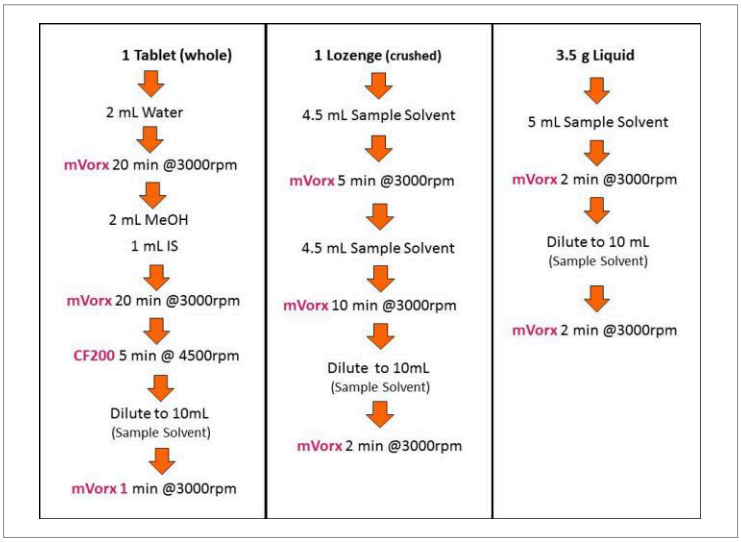

Figure 2: Schematic workflows of the on-line automated sample preparation of the three investigated product samples: tablet, lozenge and liquid ions

LC-UV conditions:

- Column: Agilent Zorbax Eclipse XDB-C18
- Injection volume: 25 µL

Tablet: Ibuprofen

Isocratic. Mobile phase 38:62 ACN/Water + 0.05% H3PO4

Flow=2 mL/min, T = 35°C, λ= 220 nm

Lozenge: Benzocaine

Isocratic. Mobile phase 70:25:5 Water/ACN/pH 3 KH2PO4 buffer,

Flow = 1.5 mL/min, T = 35°C, λ= 280 nm

Liquid: Paracetamol and other components

Gradient, Mobile phase A: 10mM pH 3 KH2PO4 buffer/ACN/MeOH 96:2:2

B: MeOH/ACN 80:20

Flow =2 mL/min, T= 25°C , λ= 215 ,220, 221, 274 nm.

Gradient: t=0 min 100% A, t= 15min 100% B, t=15.01 100% A, t= 20 min 100% A

RESULTS

Examples of the processed samples for the three tested products are shown in Figure 3.

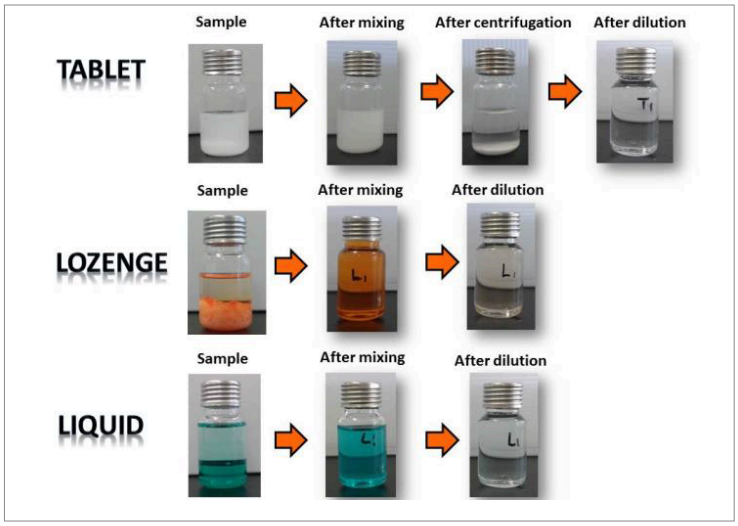

Figure 3: Processed samples for (from the top) tablet, lozenge and liquid sample preparation

Ten replicate injections were run for each of the investigated product samples to test system repeatability. Very good RSD% were obtained, ranging between 0.1% and 0.7%. Figure 4 shows examples of the LC-UV chromatograms for each product sample.

Nine replicate sample preparations of each product were run to evaluate variability of the fully automated workflows. Table 1, 2 and 3 summarise the results as retention time, analyte peak area replicates, average, standard deviation and RSD% for tablet, lozenge and liquid sample preparations, respectively.

Table 1: Results for nine replicate preparations of the tablet sample for analysis of Ibuprofen

Ibuprofen		
Sample ID	**RT**	**Area**
1	13.804	7560
2	13.875	7611
3	13.815	7552
4		
5	13.816	7531
5	13.818	7591
6	13.826	7418
7	13.829	7569
8	13.833	7562
9	13.842	7540
Average		7548
SD		55
RSD%		0.7

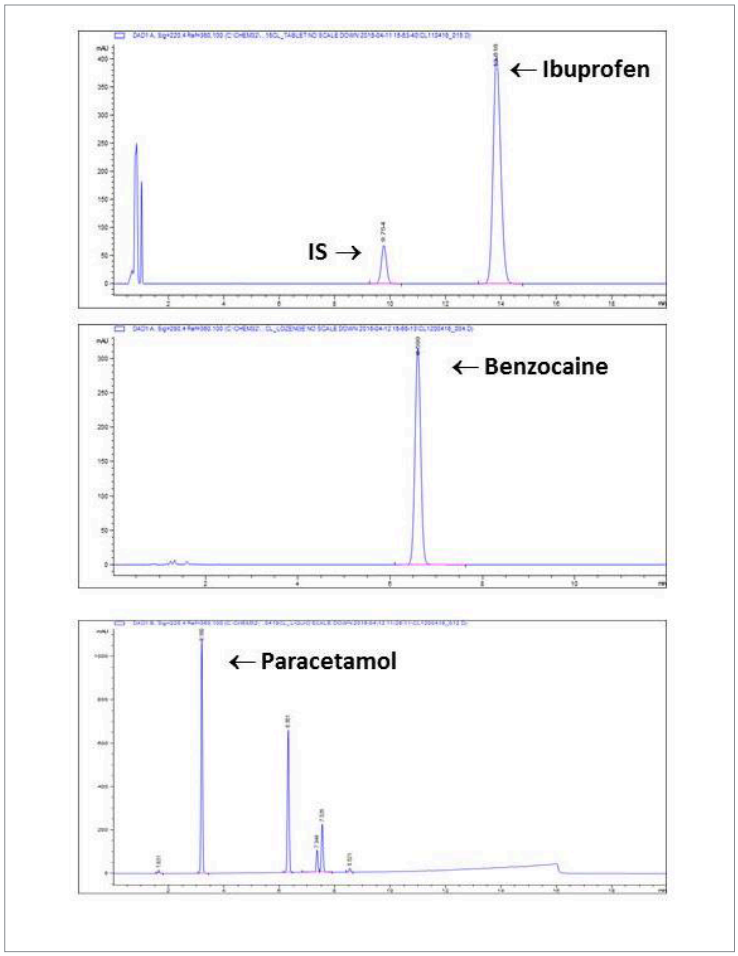

Figure 4: LC-UV chromatograms for (from the top): tablet, lozenge and liquid, respectively

Table 2: Results for nine replicate preparations of the lozenge sample for analysis of Benzocaine

Ibuprofen		
Sample ID	**RT**	**Area**
1	6.580	2898
2	6.582	2934
3	6.584	2924
4 5	6.588	2898
5	6.590	2935
6	6.591	2878
7	6.590	2973
8	6.593	2889
9	6.601	2907
Average		**2915**
SD		**29**
RSD%		**1.0**

Table 3: Results for nine replicate preparations of the liquid sample for analysis of Paracetamol

Ibuprofen		
Sample ID	**RT**	**Area**
1	3.235	3859
2	3.176	3783
3	3.180	3833
4 5	3.181	3833
5	3.187	3836
6	3.187	3800
7	3.181	3862
8	3.185	3850
9	3.182	3849
Average		**3834**
SD		**26.6**
RSD%		**0.7**

DISCUSSION

Very good RSD% (0.7%-1%) were obtained for all investigated preparations.

In addition to the analytical performances, the MPS could provide ahead preparation of each sample before LC analysis, reducing significantly the duration of the whole batch.

Sequential on-line preparation and LC analysis of the nine replicate samples took 6 hours and 27 min for the tablet, 4 hours and 55 min for the lozenge, and 3 hours and 19 min for the liquid, respectively.

CONCLUSIONS

Fully automated methods for on-line sample preparation of three product samples (tablet, lozenge and liquid) were developed in our laboratory.

The LC system offered very good repeatability (0.1%-07%).

Replicate on-line automated preparations (n=9) of the three investigated product sample proved to very reproducible (RSD% 0.7%-1%) and time saving.

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