Development of a new "Fast GC-MS" System for Multi-drug Analyses

Danny Rich[a,b], Jen Green[a], Sarah Denham[a], Linda French[a], Glenn Taylor[a], Declan P. Naughton[b]

a Hampshire County Council - Scientific Services, Hyde Park Road, Southsea, PO5 4L
b School of Life Sciences, Kingston University, Kingston Upon Thames, London KT1 2EE

Address for correspondence: Professor Declan Naughton, School of Life Sciences, Kingston University, Kingston upon Thames, Surrey, KT1 2EE, UK Email: D.Naughton@kingston.ac.uk, Telephone: +44 208 417 7097

Summary

This report details the incorporation of a recently installed "fast GC-MS" system to upgrade the existing GC-MS facility and complement the LC-MS provision for forensic analysis at Hampshire Scientific Service. Hampshire Scientific Services provides an analytical service for Coroners by screening toxicology exhibits for drugs and medicines and quantifying by LC-MS-MS where necessary. The software and hardware components were developed using a library built with standards. Optimisation steps included extension of the Agilent Deconvolution Reporting Software (DRS) which combines Chemstation, National Institute of Standards and Technology (NIST) and Automated Mass Spectral and Identification Software (AMDIS) to generate a bespoke compound mass spectral library. Secondly, a Nitrogen Phosphorous Detector (NPD) produces peak height data for standards and reference materials. The development took place according to United Kingdom Accreditation Service's standards and specific guidelines produced by the laboratory. The method is capable of analysing 20 drugs of interest with a 7 minute run time. The new system is four times quicker than the prior GC-MS system and therefore is much more cost-effective and complements current provision.

Keywords
Fast GC-MS, toxicology screening, drugs

Introduction

The horizon of forensic drug detection is rapidly changing as the pace of introduction of new "drugs" increases, providing increased challenges to the analyst. The ever-expanding range of compounds of concern includes so called legal highs (also termed new psychoactive substances), active analogues of existing drugs and more sophisticated doping agents in sport. These changes require regular updating of analytical instrumentation, along with commensurate training, in order to meet the demands of multiple analyses per sample in an efficient, cost-effective manner. Common applications of drug screening are becoming more challenging and extend to workplace drug testing[1], post mortem toxicology[2], driving under

the influence[3] and the fight against doping in sport[4]. Advances in instrument hardware bring considerable gains such as time saving in sample preparation, separation and detection as well as increases in sensitivity and throughput. Liquid chromatography and gas chromatography coupled to mass spectrometry (LC-MS and GC-MS) are fundamental tools for drug screening as an essential part of toxicological analysis[5]. For LC-MS, software advances include proprietary software for Dynamic Multiple Reaction Monitoring (Dyn-MRM) which facilitates simultaneous screening for a large number (>200) of compounds in a conventional chromatographic run[6]. By scanning for specific peaks at their expected elution times, Dyn-MRM is highly efficient with the software capability of screening as well as quantitative analyses with the option to add additional analytes[7].

Hampshire Scientific Services (HSS) carries out Toxicology Screening analysis for Coroners cases using a variety of techniques. The full portfolio includes a GC-MS screen and 2 LC-MS-MS targeted screens, a headspace GC-FID analysis for alcohol and various other volatiles, UV spectrometry, follow-up quantification by LC-MS-MS or GC-MS and occasional other wet chemistry techniques. The use of FAST GC-MS will increase capacity and amendments to the extraction method will reduce extraction time.

Fast GC-MS approaches have recently been developed and applied to analyses in toxicology[8-11]. Efficiency gains range from reduced costs per analytical run and increased sensitivity with the next generation of instruments, to considerably enhanced run capacity per day, to reduced operating costs with no requirement for additional capital equipment[12]. Fast GC-MS meets the needs of a busy forensic laboratory as it affords cost-effective rapid analysis of multiple drugs to meet emergency toxicology requirements such as in the case of treating a suspected drug overdose. Hampshire Scientific Service acquired a new FAST GC-MS system to complement their existing provision which included GC-MS and LC-MS equipped with Dyn-MRM capability. The aim of this study was to develop a new FAST GC-MS system for HSS to perform toxicological analysis on coroner's samples for a selection of analytes.

Materials and Methods

Chemicals

The standards were purchased for Hampshire Scientific Service from several suppliers: Valproic Acid, MDMA, Benzocaine, Paracetamol (Acetaminophen), Fluoxetine, Tramadol, Methadone, Amitriptyline, Nortriptyline, Mirtazapine, Carbamazepine, Sertraline, Codeine, Citalopram, Diazepam, Lamotrigine, Nordiazepam and Olanzapine were purchased from Sigma Aldrich, Gillingham, Dorset, England. AB-Fubinaca, Dihydrocodeine, Flephedrone, JWH-018, Zopiclone were purchased from LGC, Teddington, England and 7-APB from Cayman Chemicals. Boric acid, butyl acetate and tris(hydroxymethyl)methylamine were purchased from Fisher Scientific, Loughborough. All standards were certified and reagents were HPLC grade except butyl acetate which was AR grade. Blank blood samples, acquired from the NHS transfusion service, were analysed to ensure they were drug free.

Instrumentation and GC-MS Operating Parameters

An Agilent 5977A GC-MS with an Agilent 7890B GC fitted with a multimode injector port and an Agilent 7693A automatic liquid sampler were used. The chromatographic column was a DB-5MS (crosslinked and bonded Phenyl Arylene polymer, 15m x 0.25mm i.d., 0.25µm film thickness) supplied by Crawford Scientific. The oven temperature was held at 100°C for 0.25 min, then raised at 40°C/min to 325°C. Helium was used as carrier gas at a constant flow of 5.5 mL/min. The GC injection port was set at 280°C in multimode with splitless injection (purge time, 0.4 min). The system had a nitrogen-phosphorous detector (NPD) and a 5977A Mass Selective Detector (MSD). The mass detector operated in normal scan mode with an acquisition range of m/z 40-570.

Sample Preparation

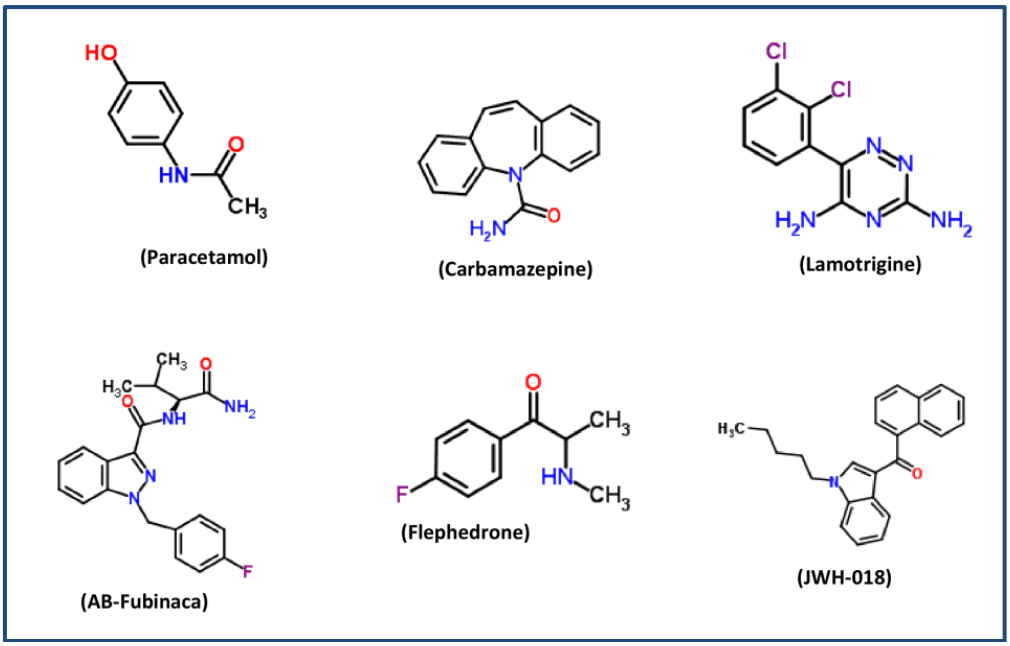
Tris(hydroxymethyl)methylamine extraction buffer (1 mL, pH=9.5, 0.5 M) was added to each intact blood sample (1mL) followed by 3 mL of butyl acetate with vortex mixing for 20±5 seconds. The mix was centrifuged at 4200 x g for 20±5 minutes or as long as necessary to separate the organic and aqueous layers. Note that complete separation was not always possible. The upper organic layer (excluding any emulsion) was transferred into a Reactivial or if additional clean-up was required, a centrifuge tube. The butyl acetate was evaporated to dryness with a stream of nitrogen on the ReactiTherm evaporation unit at a temperature not exceeding 70°C prior to analysis.

Results and Discussion

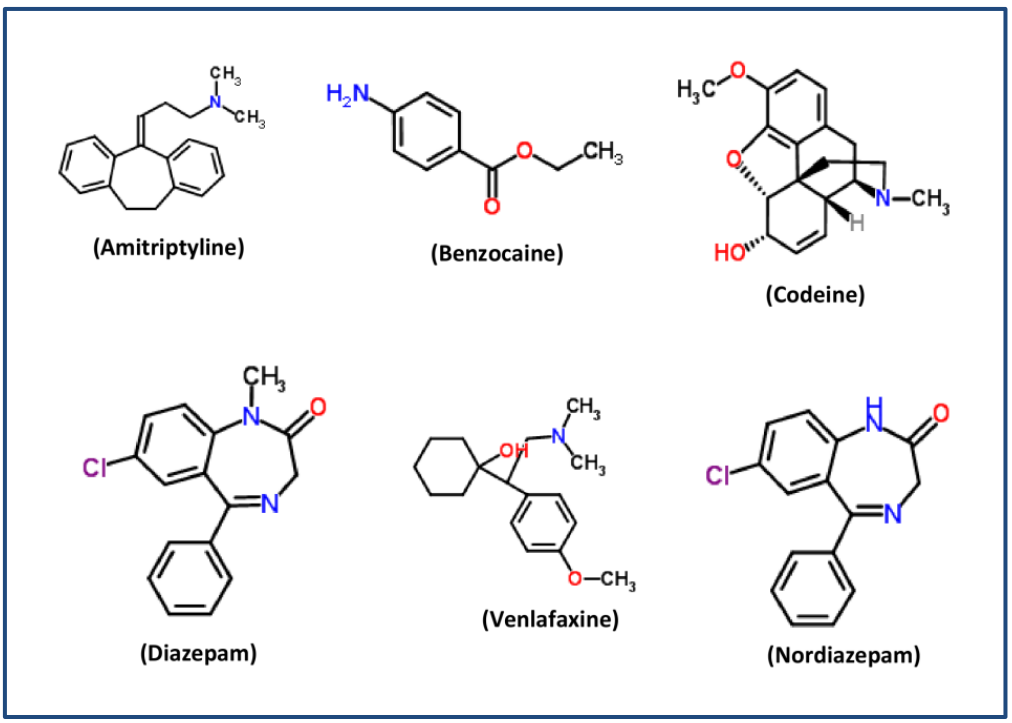
Method Development

Owing to the number and concentration ranges of drugs under investigation, drugs were grouped according to the expected therapeutic/toxic levels which dictated the range of concentrations of interest for analysis (Figure 1)[13]. For Group 1 drugs detection is desirable over the range 4 to 80 mg/L, for Group 2 drugs it is 0.1 to 5 mg/L and for Group 3 drugs it is 0.1 to 1 mg/L. During the development stages, spiking experiments in Group 1 led to solubility problems necessitating a split into two sub-groups (1.1 & 1.2). For the development the analyses were performed in duplicate for each group at every analyte concentration (Table 1) in blood over two runs with Groups 1.1 and 1.2 combined and Groups 2 and 3 together. A blank blood sample was injected between spiked sample runs. Table 1 shows the analytical parameters used; target and qualifier ions along with the retention times and lowest level detected, within the concentration range of interest, under the conditions used. In line with previous work, the optimisation was conducted to in-house specifications to meet demand as full guidelines for qualitative screening are not available[12,14].

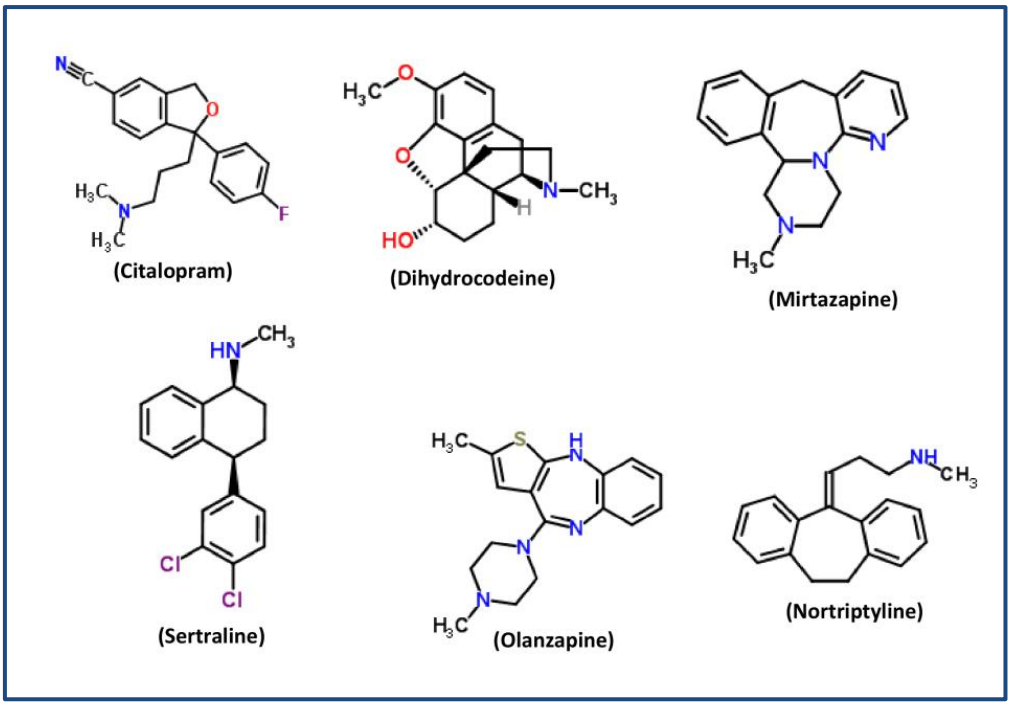
Figure 1 – Structures of Analytes grouped by their Therapeutic/Toxic Levels for Working Range Considered



Group 1 – Working Range 4-80mg/l



Group 2 – Working Range 0.1-5mg/l



Group 3 – Working Range 0.1-1mg/L

Selection of Drugs

During method development, an issue arose with the tris(hydroxymethyl)methylamine buffer as it masked the peaks for MDMA and 7-APB. A variety of alternate extraction methods were applied, including using borate and carbonate based buffers. However, all attempts led to less reliable analytical results for the range of compounds of interest. Valproic acid does not contain nitrogen or phosphorous and thus could not be detected by the detector of choice (NPD). A further issue arose with methadone as it was not resolved adequately using the software. Thus, methadone, MDMA, valproic acid and 7-APB will be screened for using other approaches such as LC-MS-MS. All of the remaining 18 drugs of interest (Table 1) could be readily detected using the method.

Table 1 – Analytical Parameters used for the Drugs in Groups

	Rt (min)	Ions (m/z)[a]	Lowest Standard[b,c]	Approx Levels significant in Postmortem Cases/Comments[d]
Group 1.1				
Carbamazapine	4.314	**263**, 193, 192, 191	4 mg/L	Therapeutic 2-9mg/L Toxic 2 mg/L Quantify if elevated or expected and absent
Lamotrigine	4.624	**185**, 187, 257, 123	4 mg/L	Therapeutic 1-15mg/L Quantify if elevated or expected and absent
Paracetamol	2.583	**109**, 151, 80, 108	4 mg/L	Therapeutic 10-20mg/L Toxic >40mg/L Quantify if elevated
Group 1.2[e]				
Flephedrone	1.438	**58**, 95, 123, 75	20 mg/L	New psychoactive substance – unknown effect levels
AB-Fubinaca	5.818	**109**, 324, 253, 254	20 mg/L	New psychoactive substance – unknown effect levels
JWH 018	6.434	**214**, 364, 307, 144	4 mg/L	New psychoactive substance – unknown effect levels
Group 2				
Benzocaine	2.268	**120**, 165, 65, 92	0.1 mg/L	It presence is usually due to having been used as a cutting agent in an illicit cocaine use
Venlafaxine	3.745	**58**, 134, 91	0.5 mg/L	Therapeutic 0.04-0.2mg/L Toxic >1mg/L Quantify if elevated
Amitriptyline	4.023	**58**, 202, 203	0.1 mg/L	Therapeutic 0.08-0.17mg/L Severe toxicity >0.25mg/L Quantify if elevated
Codeine	4.495	**299**, 229, 162, 115	0.5 mg/L	Therapeutic 0.2-0.4mg/L toxic >0.4mg/L Quantify if elevated
Diazepam	4.598	**256**, 284, 257, 255	0.1 mg/L	Therapeutic 0.06mg/L-1.4mg/L Toxic >1.4mg/L Quantify if elevated

	Rt (min)	Ions (m/z)[a]	Lowest Standard[b,c]	Approx Levels significant in Postmortem Cases/Comments[d]
Nordiazepam	4.757	**242**, 241, 269, 270	0.1 mg/L	Nordiazepam is a drug in its own right but is usually seen as an active metabolite of diazepam. The effect is additive if present with diazepam
Group 3				
Mirtazapine	4.170	**195**, 194, 196, 208	0.1 mg/L	Therapeutic 0.02–0.18mg/L Fatalities usually >1mg/L Quantify if elevated
Olanzapine	5.219	**242**, 229, 213	0.1 mg/L	If detectable on the NPD then quantify
Sertraline	4.434	**274**, 276, 262, 159	0.1 mg/L	Therapeutic 0.05–0.25 mg/L Quantify if elevated
Citalopram	4.513	**58**, 238, 42	0.1 mg/L	Therapeutic 0.045 - 0.5mg/L Fatal >3.4mg/L Quantify if elevated
Nortriptyline	4.067	**44**, 202, 203	0.25 mg/L	Therapeutic 0.09-0.25mg/L Toxic >0.25 mg/L Quantify if elevated
Dihydrocodeine	4.491	**301**, 164, 59, 70	0.1 mg/L	Therapeutic 0.07- 0.2 mg/L Fatalities usually >2mg/L Quantify if elevated

Notes:

a the **first ion** (m/z) was used as the target ion with the others acting as qualifiers

b quantification was estimated with the NPD detector by comparison to a reference medium spiked with a known concentration

c level detected is the lowest limit that was detected within the concentration range of interest in contrast to the absolute lowest limit of detection

d the above values are approximate ranges amalgamated from references [13,15] and in-house experience

e analytes in the sub-group 1.2 have recently been found at considerably lower concentrations in a post mortem case containing new psychoactive substances. Thus, it is recommended that this sub-group is screened for using targeted LC-MS-MS or Time of Flight (TOF)

Software Customisation

The analytical parameters required for the method were generated with three techniques. Firstly, the Agilent Deconvolution reporting software (DRS) combines Chemstation, National Institute of Standards and Technology (NIST) and Automated Mass Spectral and

Identification Software (AMDIS) to generate a bespoke compound mass spectral library[16]. Secondly, a Nitrogen Phosphorous Detector (NPD) produces peak data for standards and reference materials. A final source is the DRS library updates which allow analysts to add their data for dissemination. Additions to the DRS library are straightforward and the chromatographic data for analytes of interest have been added to the DRS library. In each case peak assignment was with a confidence level of greater than 80%. Varied instrument settings were trialled for sensitivity, gain factor and integration. The default settings were used with 2.5 for gain factor. A sample chromatogram read-out is shown in Figure 2, revealing that the NPD read-out is considerably cleaner than the total ion chromatogram for the signals of interest.

Conclusion

The method has been developed for qualitative screening with an indication of drug level and a focus on the selection of key drugs with efficient analysis. It is complementary to the existing local laboratory provision through other hyphenated techniques. The method is fit-for-purpose for the specified drugs in group 1.1, group 2 and group 3 to give fast turnaround of results that could have forensic immediacy in the clinical setting. For the drugs in group 1.2, it is not suitable as the levels detected are much higher than the levels of interest. The levels of detection in the suitable groups are based upon therapeutic/toxic effects. A key aspect is the ability to add to the method using the library build software.

Acknowledgement

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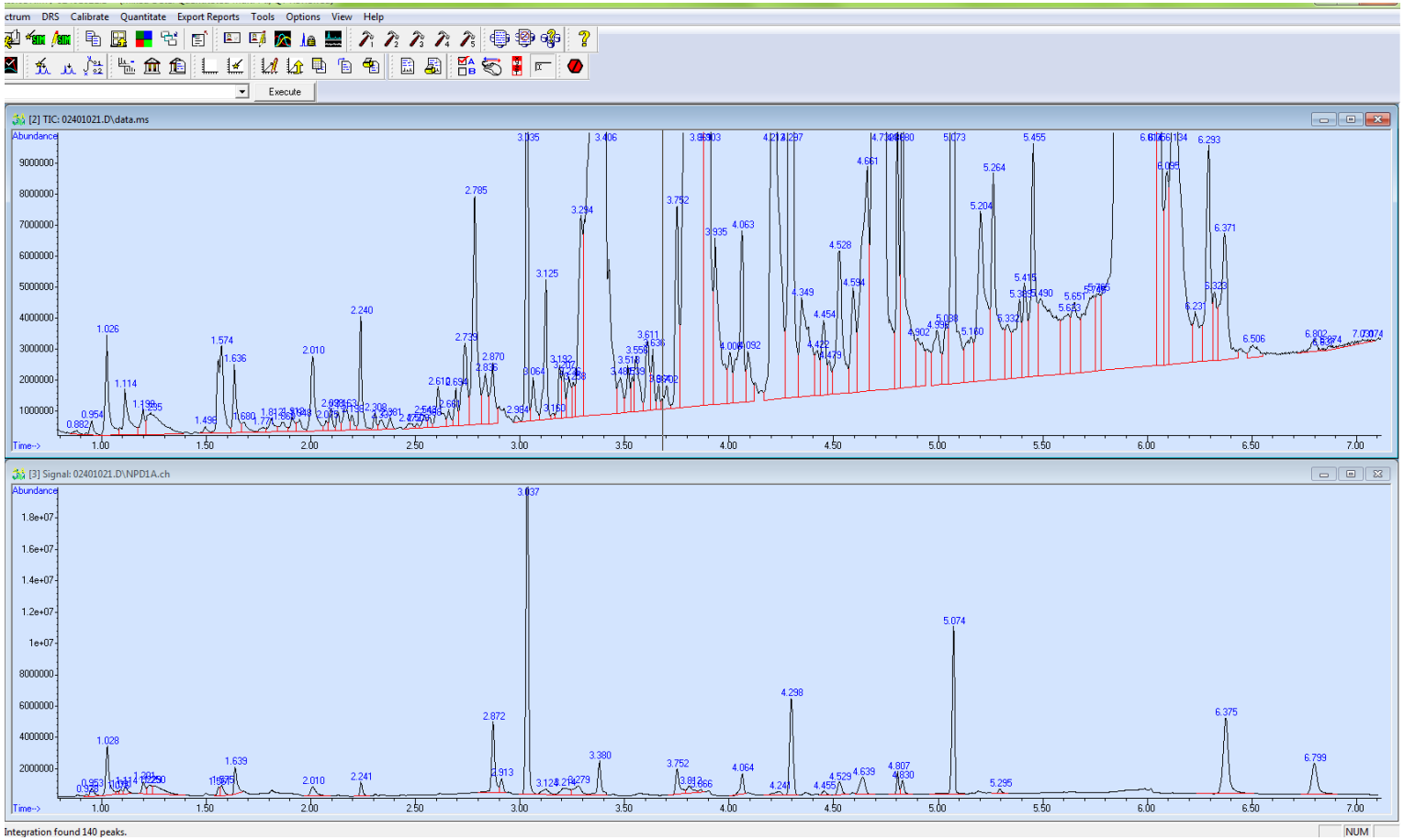
Figure 2 – Sample Chromatogram Read-Out for Group 2 (5 mg/L



Detection is by Total Ion Chromatogram (top) and NPD (bottom)
Peaks detected by NPD are within 5 minutes are: Benzocaine (2.241), Venlafaxine (3.752), Amitriptyline (4.064), Codeine (4.529), Diazepam (4.639), Nordiazepam (4.807)

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