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The design and use of a simple System Suitability Test Mix for generic reverse phase high performance liquid chromatography–mass spectrometry systems and the implications for automated system monitoring using global software tracking

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A B S T R A C T

The development of a seven-component test mixture designed for use with a generic gradient and a reversed-phase high performance liquid chromatography–mass spectrometry (RP-HPLC–MS) system is discussed. Unlike many test mixtures formulated in order to characterise column quality at neutral pH, the test mixture reported here was designed to permit an overall suitability assessment of the whole liquid chromatography–mass spectrometry (LCMS) system. The mixture is designed to test the chromatographic performance of the column as well as certain aspects of the performance of the individual instrumental components of the system. The System Suitability Test Mix can be used for low and high pH generic reverse phase LCMS analysis. Four phthalates are used: diethyl phthalate (DEP), diamyl phthalate (DAP), di-n-hexyl phthalate (DHP) and dioctyl phthalate (DOP). Three other probes are employed: 8-bromoguanosine (8-BG), amitryptyline (Ami), and 4-chlorocinnamic acid (4-CCA). We show that analysis of this test mixture can alert the user when any part of the system (instrument or column) contributes to loss of overall performance and may require remedial action and demonstrate that it can provide information that enables us to document data quality control.

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1. Introduction

The development of a system suitability protocol for RP-LCMS dates back to the creation of a GlaxoSmithKline (GSK) high quality screening collection through amalgamation of the SmithKline Beecham (SB) and GlaxoWellcome (GW) heritage collections [1]. A quality control project accompanied the creation of this new high quality screening collection, facilitating removal of 'bad' samples which were either impure or structurally incorrect. This project employed LCMS as its first pass method of analysis and to have confidence in the analytical data for a project of this scale and throughput (1.4 million total samples analysed over 24 h per day, seven days per week for 18 months), there was a critical requirement for compliance to a minimum standard for both chromatographic performance as well as the performance of all instrumental equipment, termed system suitability [1]. System suitability as used here, means the use of a solute mixture, in conjunction with as generic a set of HPLC–MS method conditions as possible, to confirm adequate separation and detection performance using these generic conditions for any subsequent sample analysis. Previously, other published work has described using a versatile, carefully chosen test mixture to confirm method performance of a more targeted system, aimed at compounds eluting within a more defined property range [2]. This work also adopted a rudimentary system self-testing approach which we felt had considerable scope and we develop further, particularly for the chromatographic component of the system, in this work. The first solute mix (Mix A) consisted of three phthalates (DEP, DAP and DOP) and 8-bromoguanosine (8-BG) with the majority of checks being visual, plus one calculation of resolution between the DEP and DAP peaks. The primary intention was to demonstrate sufficiently reproducible and trustworthy 'generic' operation of the method, such that it was capable of eluting everything that may be present in the sample, even components as hydrophobic as di-octyl phthalate (DOP). The adoption of the system suitability protocol ensured that the analytical data used to make decisions as to entry into the collection based on confirmed identity and minimum purity, was of sufficiently high quality and analytically assured.

The benefits of using the System Suitability Test Mix (SSTM) were such that it has subsequently been adopted for all routine applications in Discovery LC–MS systems within GSK, spanning open-access and expert systems and including systems that quality control the screening collection as it is enhanced with internally and externally acquired compounds.

Subsequent revisions of this first method have been necessary to provide flexibility and a wider range of options for analy-

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sis. Any intended generic method must first address a very wide lipophilic range. This is only realistic by using gradient elution RP-HPLC. Typically, systems employed in drug discovery utilise low pH mobile phases: under these conditions, the high proportion of lipophilic compounds that are also bases can be conveniently chromatographed as their protonated form, as ammonium salts or as base-pairs. Low pH also promotes positive ionisation of these bases in the MS. Trifluoroacetic acid (TFA) is found to be a useful chromatographic modifier, but interference with ionisation in the MS can occur in the negative ion mode. As scanning in negative ion mode is part of our GSK analytical protocol, this favours the use of formic acid and this is the preferred way to lower the method pH for our LCMS applications. Due to the demand for a fast turn-around of a large number of samples, gradients are chosen to be very short, but as shallow as possible. For this reason, short columns, often of narrow-bore, are operated with as high a linear flow that can be reliably tolerated by the chromatographic system. Injectors, columns and detectors operate under demanding conditions, and so a simple, standardised test that validates the correct performance of the chromatographic system is very useful.

We discuss in this communication the design and use of a seven-component mix that enables the user to ensure that system performance is adequately maintained. This includes checks of gradient delivery accuracy, injector function, and that the detector(s) are performing adequately. The holistic nature of the test is emphasised by the fact that most of the probes are used for more than one of these tasks.

Finally, we report the use of software written in-house (Verify) to extract relevant metrics, both chromatographic and spectral, across multi-vendor instrument configurations. If the mix is incorporated into daily system qualification routines, we show how this can be used to significantly identify operational intolerances and hence, ensure data quality control is maintained.

2. Experimental

2.1. Materials

8-Bromoguanosine (8-BG, CAS 4016-63-1), nortryptyline hydrochloride (Nor·HCl, CAS 894-71-3), amitryptyline hydrochloride (Ami·HCl, CAS 549-18-8), 4-chlorocinnamic acid (4-CCA, CAS 1615-02-07), di-ethyl phthalate (DEP, CAS 84-66-2), di-amyl phthalate (DAP, CAS 131-18-0), di-n-hexyl phthalate (DHP, CAS 84-75-3), di-octyl phthalate (DOP, CAS 117-84-0), dimethyl sulphoxide (DMSO), formic acid (98/100) and trifluoroacetic acid (HPLC Grade) were obtained from Sigma–Aldrich, Gillingham, UK. Research samples used in the course of this work were made available from current synthetic programmes at GSK, Stevenage, UK, either as dry solids or as solutions in DMSO. HPLC grade acetonitrile (MeCN) and *N*,*N*-dimethylformamide (DMF) was purchased from Fisher Scientific UK Ltd., Loughborough, UK. Water was purified through an ELGA water purification system (High Wycombe, UK). HPLC columns were employed as indicated in the text and were obtained from the appropriate manufacturers.

2.2. Instrumentation

The initial testing by LCMS of the new system suitability mix was performed on Agilent HP1100 and 1200 Series instruments equipped with diode-array detection (Agilent Technologies, Waldbronn, Germany). The static mixers were replaced with A-330 Semi-Prep filters (Anachem, Luton, UK) to provide a low dwell volume configuration. Mass spectral data were acquired on an Agilent 1100 MSD operating in full scan positive ionisation mode. The operating software was ChemStation version 10.1. After the initial testing phase the test mix was put out for testing on multiple sites on standard OA LCMS equipment. Here Acquity UPLC systems with either a ZQ or SQD mass spectrometer (Waters Corporation, Milford, USA) were used with the operating software being Masslynx 4.1. The evaporative light scattering detectors (ELSD) tested were the PLS1000 and PLS2100 from Polymer Labs (Church Stretton, UK), the Waters Acquity ELSD and the Sedex 60 and 75 (Sedere, Alfortville, France).

2.3. Test mix preparation

Various test mixtures were used and refined to provide the maximum information from the simplest mixture. The initial test mix (Mix A) had 4-CCA, Nor and DHP (1%) added to the original four components to form Mix B1. The nortryptyline was found to be unstable in the presence of the other components and was replaced with amitryptyline to form Mix B2.

Subsequent test Mixes B1, B2 and B3 were prepared so that the concentrations of the components fell within the linear range for all the HPLC and UPLC detectors that we used in the various LCMS environments. The composition of B1 and B2 was: 8-BG 0.70 mg/ml, Nor·HCl (B1) or amitryptyline·HCl (B2) 1.17 mg/ml, 4-CCA 0.66 mg/ml, DEP 5.52 mg/ml, DAP 8.38 mg/ml, DHP 0.09 mg/ml and DOP 8.12 mg/ml. Mixes B1 and B2 were prepared in DMSO/MeCN (1:1, v/v). The composition of Mix B3 was: 8-BG 0.50 mg/ml, amitryptyline·HCl 0.8 mg/ml, 4-CCA 0.4 mg/ml, DEP 3.0 mg/ml, DAP 4.5 mg/ml, DHP 0.2 mg/ml and DOP 5.5 mg/ml. Mix B3 was prepared in DMF. Based on a knowledge of the measured UV responses of known concentrations of DEP and DAP, the anticipated peak area ratio (PAR) of Mixes B2 and B3 were 1.09 and 1.10 at 254 nm respectively. Test mixes were prepared in bulk, dispensed into 2 ml amber capped vials and stored at 4 °C.

2.4. Use of test mixtures

Mix A was initially used to test the accuracy of a number of available HPLC autosamplers. The injection parameters were selected to be appropriate for the instrument, autosampler, column and gradient combinations.

Mixes B1 and B2 were designed to give roughly equal peak heights (except for DHP) at 254 nm on a 3.9 min gradient of 0–100% MeCN (+0.1%, v/v formic or trifluoroacetic acids) on a 50 mm × 4.6 mm column of Waters 3.5 μm SunFire C18. A typical trace from the analysis of test Mix B1 is shown under the above conditions in Fig. 1. Other systems may provide chromatograms of slightly different appearance. Injection volumes were adjusted to provide a peak height not exceeding 1.5 AU for the largest peak at 254 nm to maintain linearity of the detector signal. This normally allowed the 1% DHP component to be easily visible at absorbance levels up to 10–15 mAU. Mix B2 was used by an international panel of GSK chromatographers to demonstrate its utility on a variety of open access (OA) and QA systems. As a result of experience gained during a continuing programme of progressive improvements, Mix B3 was prepared in DMF at a slightly lower concentration, and the DHP concentration was adjusted to represent 1% of the total area of the whole chromatogram and this mixture was used in the implementation of Verify [3].

2.5. Verify software

Verify software, written in house, was designed to analyse data from report files generated using Waters OpenLynx (*.rpt) or Agilent AEVGen software (*.aev). Verify [3] was designed to perform regular system performance checks. Initially these checks were run weekly, but with the introduction of greater automation a daily pro-

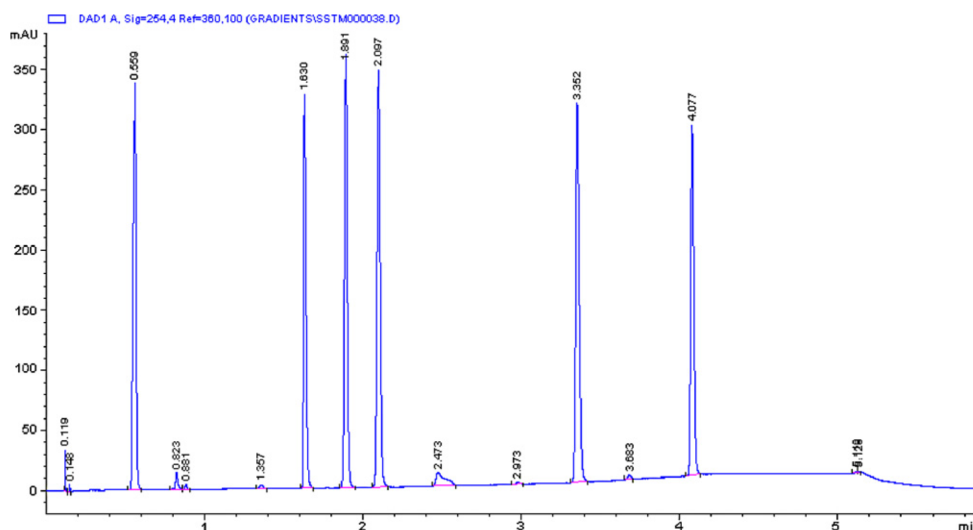

Fig. 1. Chromatogram of 0.5 μl aliquot of Test Mix B1. Column: 50 mm × 4.6 mm × 3.5 μm SunFire C18 (Waters Corporation). Mobile phase "A": deionised water + 0.1% (v/v) trifluoroacetic acid. Mobile phase "B": acetonitrile + 0.1% (v/v) trifluoroacetic acid. %"B": 0% (0.0–0.1 min) to 100% over 3.9 min, held at 100% for 1.0 min, returned to 0% over 0.2 min, 0% thereafter. Flow rate: 3.0 ml/min. Detection: 254 nm. The identity of the major peaks is (in order of elution): 8-BG, Nor, 4-CCA, DEP, DAP, and DOP. The peak due to the 1% relative area component DHP is seen at 3.683 min, and the peak associated with interaction between Nor and 4-CCA is seen at 2.473 min.

tocol was established. These checks capture data in a manner that allowed easy interrogation of trend data.

3. Results and discussion

3.1. Selection of probes

The starting point for test mix design was the wide variety of column testing procedures available to the chromatographer. Typically such tests address the column efficiency, selectivity, and any undesired activity towards acids, bases, polar compounds and metal chelators. Our purpose was to ensure adequate performance in unbuffered organic/aqueous gradient systems, using only acid to adjust the aqueous component to low pH. Under these conditions, the most common adverse activity that we have observed is for strongly basic compounds, arising from unwanted silanol interactions, even on many late generation end-capped, or hybrid materials. Many of the available column tests have recently been reviewed [4] and the interested reader is referred to this source for an excellent discussion of the history of this topic. We wished therefore to design a test that had a somewhat different emphasis: that of monitoring the suitability of the complete chromatographic system under the conditions with which it is most commonly used.

Since our experience with formic and TFA based systems was that even when present predominantly as the protonated form or an ion-pair, basic compounds could potentially display poor peak shapes, we first required a sensitive basic probe. We examined a number of candidates, including pyridine (pKa = 5.2), benzylamine (pKa = 9.3), quinine (pKa = 8.7), nortriptyline (Nor) (pKa = 9.7) and amitryptyline (Ami) (pKa = 9.4), and found the latter two to be by some way the most indicative of poor column performance. Poor column performance was indicated by some or all of these features: increased retention, tailing, asymmetry or an apparent loss of peak area due to the difficulty in integrating peaks with such an extremely asymmetric shape. These two candidates, nortriptyline and amitryptyline, were selected for further study. Although we had very few instances of poor chromatography of acidic probes, which would generally run as the conjugate acid, it was decided to incorporate an acidic probe in anticipation of the occurrence of any unusual effects due to acidic compounds, with residual basic activity in some base-deactivated C18 materials. In accordance with earlier work [5], 4-chlorocinnamic acid (4-CCA) (pKa = 3.7) and 4-nitrobenzoic acid (4-NBA) (pKa = 3.4) were examined as potential acidic probes. They both chromatographed well under all conditions tested, and 4-CCA was chosen as it co-eluted with other mix components in fewer circumstances than did 4-NBA. Some metal chelators, including 2-hydroxy-5-methylbenzaldehyde, 2,3-dihydroxynaphthalene, and quinine derivatives were also tested, but with the commercial columns employed we found no evidence of unwanted activity.

There are many tests in the literature that represent descriptors of column selectivity, enabling valuable means of column classification [6–17]. Whilst knowledge of column selectivity is invaluable when designing and optimising specific separations, these data are less informative about the range of undesired activities potentially observable with columns of generally similar overall selectivities. Column selection for generic gradient work with unknown compounds is therefore largely dictated by issues such as residual activity, efficiency, and robustness rather than specific selectivity attributes. For this reason the minimal choice of basic and acidic probes were considered sufficient for monitoring the activity of the chromatographic system at low pH.

All our instrumentation must be capable of delivering rapid gradients and running reliably during periods of unattended operation, therefore the user must ensure that the gradient is being correctly delivered to the column. We have determined empirically that the majority of NCEs addressed by our generic reversed-phase gradients have lipophilicities intermediate between those of 8-bromoguanosine (8-BG) (cLog $P = -2.27$) and di-octyl phthalate (DOP) (cLog $P = 8.97$). We have developed generic gradient systems such that elution of 8-BG and DOP mark respectively the delivery to the exit of the column of the start and end of the programmed acetonitrile gradient. The retention times of these two probes may thus be used to confirm correct operation of the pumps and gradient delivery. In addition, any drift in the 8-BG retention time can be used to indicate changes in dwell volume between different systems, or disturbances due to injection of too much and/or too strong an eluotropic sample solvent, which may occur for example in generic gradient analysis of some reaction mixes.

Chromatographic systems require column changes once the performance of the column has deteriorated to a particular point. In practical terms this may be quite difficult to define with a number of systems globally that have diverse local histories, and so

it is desirable to define practicable and self-consistent criteria to trigger system investigation, thus ensuring a high standard of data integrity. The end of column lifetime may also be flagged by loss of column efficiency due to the formation of column voids. Efficiency cannot be directly measured in a gradient, but resolution can and for this reason two neutral probes (DEP and DAP) were included in the test mixture. The intention here is to use these two neutral probes to measure resolution between this specific solute pair and ultimately relate this to the peak capacity of the separation method. These homologous probes were selected because they will always elute within a gradient bound by 8-BG and DOP. In practice, the resolution between these compounds, taken with satisfactory peak shape, i.e. as near symmetrical as possible, can be used to specify and confirm whether a given column is still fit for purpose.

Additionally, the extinction coefficients of DEP and DAP can be used to predict the peak area ratios of gravimetrically prepared solutions of the mix. We have observed by such means that in certain circumstances a number of sampling devices may fail to deliver a representative bolus of test or sample solution to the column. Significant discrimination against the more lipophilic components of test mixtures has been noted in many instances, particularly in early designs of some commonly used autosampling devices. The DEP/DAP peak area ratio is thus an important metric used to indicate that representative analysis is obtained.

The final probe used in the mixture is dihexyl phthalate (DHP), which is added at a level equivalent to 1% in the purity profile of the test mix. This provides a means to check that the detection system continues to enable components present at low levels to still be easily detectable.

All seven components are detected by MS. The mass accuracy settings for the MS data are user defined. Typically we set a value of $\pm$0.2 amu window for our OA LCMS systems that use single quadrupole MS detectors. The MS peak widths should be no greater than three times the peak width of the DAD peaks.

The mix was run on a number of different GSK systems to optimise mix concentration, and test mix stability. During this exercise a minor peak appeared in test Mix B1 that contained the secondary base nortriptyline, but not with the tertiary probe amitriptyline in B2. Amitriptyline was selected as the base probe, and Mix B2 used as the preferred formulation for further studies. No degradation of B2 was observed over a six month period during the testing when amber glass vessels were used. Some evidence of degradation of the mix on exposure to light was seen if the test mix is stored in clear glass vessels. Although the mix is chemically stable, it was noticed that repeated piercing of a septum could result in ingress of moisture owing to the hygroscopic nature of DMSO. This in turn promoted a slow precipitation of the more lipophilic phthalates, causing the DEP/DAP peak area ratio to rise. In order to prevent this effect, the concentration of major components was reduced and the matrix solvent was changed to DMF, resulting in the Mix B3.

Typical chromatograms obtained from Mix B3 are seen in Figs. 2 and 3. The analysis conditions used were a 1.5 min gradient of 3–100% MeCN/0.1% (v/v) formic acid with a hold for 0.4 of a minute at 100% MeCN. Flow rate 1 ml/min. The column was a 50 mm × 2.1 mm × 1.7 μm Acquity BEH C18 (Waters). The column temperature was set to 40 °C. The injection volume was 0.2 μl.

3.2. System suitability parameters

Several parameters may be used to demonstrate that the chromatographic system as a whole continues to be fit for the intended purpose. As well as monitoring the column performance, we can monitor the performance of the injector, pumps, and detector and so together provide an overview of System Suitability. The user may define the minimum performance values or acceptance criteria according to local needs or business requirements.

3.2.1. Retention time and peak shape of 8-BG

We have found 8-BG to be a useful probe for fast-gradient work as, for systems with minimal dwell volumes, its elution corresponds closely to the point at which the first solvent in the programmed gradient is delivered to the detector. Shifts in peak shape and retention time may indicate a number of minor undesirable effects, sometimes due to a loss of volume integrity as a result of leaks or column bed compression, otherwise indicative of incomplete re-equilibration of the gradient to starting conditions. A change in peak width or symmetry, or evidence of peak splitting could indicate that the injection solvent has too high a relative eluotropic strength for the system employed (this may also occur if the flow rate is increased in order to make the gradient more shallow), or that the injection volume is too large. In both cases peak distortion is due to "surfing" on a bolus of injection solvent that has not fully dispersed and mixed with mobile phase and therefore can show disrupted chromatographic peak integrity by the time of elution from the column.

The 8-BG peak is also a useful diagnostic in a mass spectrum – the strength and isomeric characteristics of the molecular ion can be used as an indicator of satisfactory MS performance. Loss of ionisation in negative ion mode is indicative that the source requires cleaning.

3.2.2. Retention time and peak shape of amitryptyline

The amitryptyline peak serves as a marker of column performance. The parameters for retention time, peak width and symmetry may be used to monitor the ageing of a column and thus to be a user-defined criteria, highlighting the point at which a new column is required. With loss of ligand from a C18 column, amitryptyline retention might decrease very slightly in a rapid gradient run. This would only be expected if the surface silanols were heavily end-capped and access to them were sterically hindered. Otherwise, it is more likely that exposure of silanols during ageing will promote ion-pair reactions between the silica surface and this strongly basic probe, leading to increased retention, peak broadening and asymmetry. We have observed these features with several columns, and indeed all may be used as metrics to ensure analysis quality control. An example of the performance of a new and aged column is seen in Figs. 2 and 3 respectively. Note the dramatic change in the retention characteristics of the amitryptyline peak.

3.2.3. Retention time and peak shape of 4-CCA

This probe is present to check for the absence of basic sites. We have found little evidence for serious build-up of basic sites on-column during use, even after the columns have been repeatedly used in open-access mode for the analysis of strongly basic materials.

The molecular ion of 4-CCA can be used to monitor MS performance in negative ion mode (this is available as all mass spectra are collected in positive/negative switching mode). Loss of molecular ion response is a good indicator for when the source requires general cleaning.

3.2.4. Resolution between DEP and DAP

The two phthalates were selected because they would be certain to elute from the column during delivery of any combination of instrument, column and gradient envisaged for generic high throughput work. That is to say that DEP is sufficiently retained so that it could not elute during the quasi-isocratic period representing dwell time at the start of the gradient, and no conditions were anticipated that would require the concentration of MeCN to

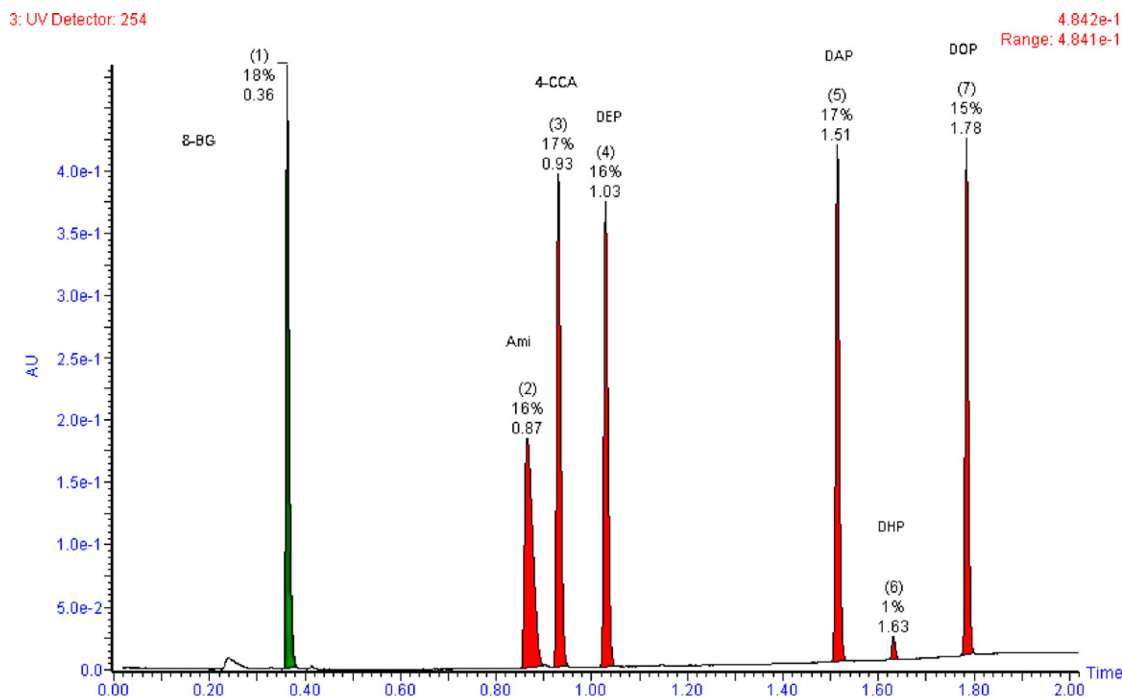

Fig. 2. Chromatogram of 0.2 μl aliquot of Test Mix B2. Column: 50 mm × 2.1 mm × 1.7 μm Acquity BEH C18 (Waters Corporation). Mobile phase "A": deionised water + 0.1% (v/v) formic acid. Mobile phase "B": acetonitrile + 0.1% (v/v) formic acid. %"B": 3–100% over 1.5 min, held at 100% for 0.4 min, returned to 3% over 0.1 min. Flow rate: 1.0 ml/min. Column temperature 40 °C. Detection: 254 nm. The identity of the major peaks is (in order of elution): 8-BG, amitryptyline, 4-CCA, DEP, DAP, DHP and DOP.

reach 95–100% before DAP would be eluted. The resolution $R_{\mathrm{DEP/DAP}}$ between these two probes is therefore representative of the gradient peak capacity of the column under the conditions used. $R_{\mathrm{DEP/DAP}}$ was calculated using the conventional equation:

$$R = \frac{(2.35/2)(t_{R(b)} - t_{R(a)})}{w_{50(b)} + w_{50(a)}}$$

where $t_{R(b)}$ and $t_{R(a)}$ are the respective retention times of DAP and DEP and $w_{50(b)}$ and $w_{50(a)}$ are the respective peak widths at half-height of DAP and DEP.

In Fig. 1, DEP and DAP eluted 1.26 min apart with a resolution of 28. The value of resolution is therefore a particularly important diagnostic as it provides an almost instant check on the resolving power of the whole chromatographic system. Rather than adopt-

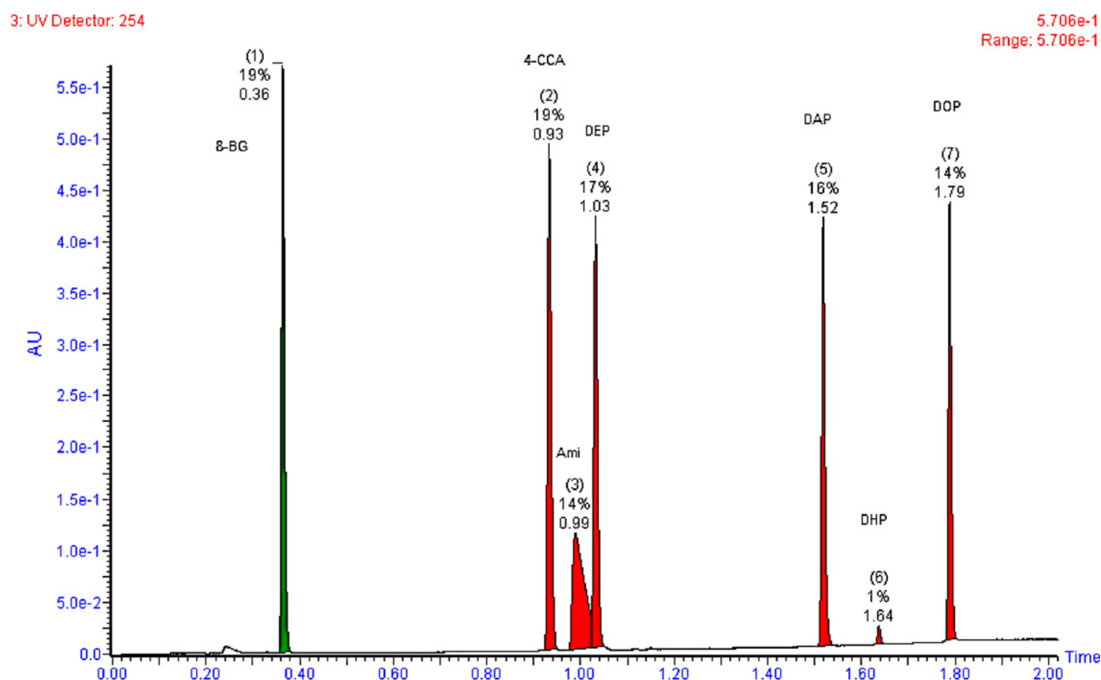

Fig. 3. Chromatogram of 0.2 μl aliquot of Test Mix B2. Column: 50 mm × 2.1 mm × 1.7 μm Acquity BEH C18 (Waters Corporation). Mobile Phase "A": deionised water + 0.1% (v/v) formic acid. Mobile phase "B": acetonitrile + 0.1% (v/v) formic acid. %"B": 3–100% over 1.5 min, held at 100% for 0.4 min, returned to 3% over 0.1 min. Flow rate: 1.0 ml/min. Column temperature 40 °C. Detection: 254 nm. The identity of the major peaks is (in order of elution): 8-BG, 4-CCA, amitryptyline, DEP, DAP, DHP and DOP. Ami peak has shifted in retention time due to ageing (possible exposure of silanols) of the column.

ing a resolution figure that just relates to a specific elution window within the gradient, we can translate this performance 'figure of merit' into a peak capacity value (PC) for the chromatogram. This is a simple and increasingly accepted way to measure gradient method performance. Arbitrary acceptance values can be set for peak capacity, for each method, and when the column deteriorates to a point where this value is not achieved then a new column would be indicated. Monitoring column deterioration with the use of peak capacity has shown significant variability in the robustness of different commercial brands of ODS-type columns. Knowledge of this informs future optimisation of purchasing strategies and global methods.

3.2.5. Monitoring the DEP/DAP ratio

A number of autosamplers of different designs were tested with Mixes A and B2 using aliquots designed to produce DEP and DAP peaks of heights <1.5 AU. The DEP/DAP peak area ratio (PAR) at 254 nm, was observed in some circumstances to be more than 25% in excess of the anticipated value of 1.09. Generally this occurred with old injectors, experimental injector designs, when the injection parameters had been set so as to telescope gradient injections or to reduce effective dwell volumes. In extreme circumstances the PAR exceeded a value of 2 owing to substantial loss of DAP in the injector. Generally the injectors caused little or no discrimination, and Mix B3 is currently used to confirm representative sampling from injectors used on workhorse OA and QA systems.

3.2.6. Integration of DHP

At the drug discovery stage, adequate estimates of sample purity can be assured if the user is confident of seeing the presence of low level impurities. Integration of DHP, present at approximately 1% of the total purity profile of the test mix provides assurance this is the case.

3.2.7. Retention time of DOP

Depending on the column and gradient employed, the DOP peak emerges either at or close to the point at which a full acetonitrile gradient leaves the column. Good design of open access systems for NCEs includes a short isocratic hold at the top of the gradient, and DOP elution during this ensures stable gradient delivery has been completed, and that the method design is appropriate for confident elution of hydrophobic compounds. We have not observed significant drift of this probe during routine operation of our systems. The DOP peak is also a valuable tool for the mass spectroscopist, the presence of both the molecular ion and a dimeric DOP species indicate that the MS source is operating correctly. Loss of these ions can be an indicator that either the source requires cleaning or that the eluent has been prepared incorrectly and there is insufficient formic acid present to aide in the ionization process.

3.3. System suitability software

All the parameters indicated in the preceding section must be calculated automatically, calculated manually, measured or observed. Ideally, all these measurements, observations and calculations would be done automatically. The Verify software splits the performance measures into two categories:

1. Chromatographic
2. Spectroscopic

To obtain chromatographic measurements the software parses the time–intensity (X,Y) data. These data are then used to calculate peak width at half height, using this we can calculate resolution, peak capacity and signal to noise for the DHP peak. The software also interrogates the report files for peak retention time and peak area.

The mass spectral data are parsed to check that the monoisotopic molecular weights that represent the seven test mix probes are observed with the correct retention times.

These data are automatically collated daily and stored within a *.csv file. This file can then be interrogated for trends. For more information regarding the methodology used in Verify and the data produced and how they can be used please refer to reference [3].

3.4. System suitability test metrics

To date, this test, implemented together with 'Verify' automated monitoring software, has been used to monitor the performance of up to 20 instruments, running the same methodology, across our global organisation. In the year since it has been in place these systems have been responsible for the 'qualified' analysis of >500,000 compounds. Each system will run the SSTM test daily, so approximately 7000 analyses per annum will be of the SSTM itself. Generally retention time accuracy is monitored to within 2% of the set value and a minimum performance capability in terms, of gradient peak capacity is set. If this drifts out of this specification it will be 'flagged' by the software monitoring system and the cause investigated. Using this protocol, typical column lifetimes (when the column performance drift is not correctable and due to genuine loss of column performance) are 10,000–20,000 samples. It is expected that this monitoring system and test will be introduced to many more systems going forward and so should be responsible for monitoring the performance of many millions of samples in the future.

4. Conclusions

We have reported a simple seven-component System Suitability Test Mix and software for automated test processing that enables holistic monitoring of all major components of RP-LCMS systems.

Using runs as short as 2–3 min, it is possible to confirm that the pump flow delivery, autosampler, column performance and residual activity, UV and MS detectors all continue to perform to a standard known to be fit for the required purpose. The test has been used to date to monitor over 500,000 analyses across 20 systems around the world. A test such as this could form the basis of a long-term comparison parameter to compare system similarity and method performance between systems and different laboratories, or to evaluate the ultimate separation performance of a system-column combination. The System Suitability Test Mix uses readily available chemicals, is easy to prepare, and appears to be stable if stored at 4 °C in an amber/brown vial.

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