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β-Diketones: Peak shape challenges and the use of mixed-mode high-performance liquid chromatography methodology to obtain fast, high resolution chromatography

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ABSTRACT

Historically, indirect methods have been used for the HPLC analysis of β-diketone compounds because of the very poor peak shapes and resolution obtained on conventional HPLC stationary phases. In this paper we demonstrate that it is possible to obtain good peak shapes for underivatised β-diketone compounds, in a simulated reaction mixture, using only conventional mobile phases with mixed-mode stationary phase HPLC columns. Optimum conditions were obtained using the mixed-mode reversed-phase strong anion exchange column Primesep B, supplied by SIELC Technologies, with a 0.1% aq. TFA/MeOH gradient method and a column temperature of 55 °C.

1. Introduction

As a class, β-diketones have been widely investigated in the literature for a diverse range of uses. These virtually all involve the formation of metal chelates or other derivatives, whether it is for purely structural and spectroscopic [1,2] or photochemical [3] studies, metal ion extraction from aqueous media [4] or purely as a means of analysing metallic species by gas [5] or liquid chromatographic [6,7] methods. However, the HPLC analysis of the β-diketone compounds themselves has always proved difficult, being characterised by very poor peak shapes and injection reproducibility. This has generally been attributed either to keto-enol tautomerism [8] or secondary retention effects caused by active silanol groups and/or associated trace metal ions present on the silica surface [9]. The general approach to solve this problem has usually been to preform a more chromatographically amenable metal chelate as above. Moriyasu et al. [8] prepared a difluoroborane derivative whilst Blanchette et al. [10] formed quinoxaline derivatives by reaction with o-phenylenediamine. Smith et al. [9] adopted an alternative approach by preparing the metal chelate in situ by the inclusion of metal ions in the mobile phase.

We required a chromatographic method to analyse a simulated reaction mixture containing the reactants 3-hydroxy-5-isocyanobenzonitrile and 4-chloroheptane-3,5-dione (Fig. 1 compounds **1** and **2**, respectively), the product 3-isocyano-5-(2-oxo-1-propionylbutoxy)benzonitrile (Fig. 1 compound **3**) and by-products 2-oxo-1-propionylbutyl propionate, 3-isocyano-5-(2-oxobutoxy)benzonitrile and 3-[(2,5-diethyl-4-propionyl-3-furyl)oxy]-5-isocyanobenzonitrile (Fig. 1 compounds **4**, **5**, and **6**, respectively). Of these compounds **2**, **3** and **4** were all β-diketones. The extra requirement for a 5 min method run time meant that good peak shape was essential for all the components in the reaction mixture. Before adopting the approaches of Moriyasu et al. [8], Smith et al. [9] or Blanchette et al. [10], we evaluated several of the more modern stationary phases that have become available in the past few years.

2. Experimental

2.1. Chemicals and reagents

Water was purified using a Milli-Q water purification system. Chromasolv HPLC grade acetonitrile (ACN) and methanol (MeOH), and formic acid (HCOOH) were supplied by Sigma–Aldrich. Extra pure (99%) trifluoroacetic acid (TFA) was supplied by Acros Organics. Orthophosphoric acid (H_3PO_4), potassium dihydrogenphosphate (KH_2PO_4) and sodium hydroxide pellets (NaOH) were supplied by Fisher Scientific. Drug related products were produced in-house by Pfizer Global R&D.

2.2. Apparatus

The HPLC system used was an Agilent 1100 comprising of a binary pump (G1312A), column heater (G1316A), injec-

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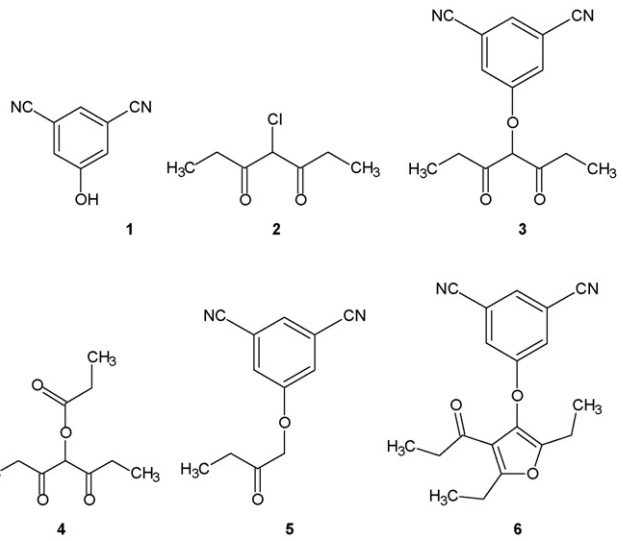

Fig. 1. Components of simulated reaction mixture: (**1**) 3-hydroxy-5-isocyanobenzonitrile, (**2**) 4-chloroheptane-3,5-dione, (**3**) 3-isocyano-5-(2-oxo-1-propionylbutoxy)benzonitrile, (**4**) 2-oxo-1-propionylbutyl propionate, (**5**) 3-isocyano-5-(2-oxobutoxy)benzonitrile and (6) 3-[(2,5-diethyl-4-propionyl-3-furyl)oxy]-5-isocyanobenzonitrile.

tor (G1367A), diode array detector (G1315B) and degasser unit (G1379A) operating with Chemstation software (Version A.09.03). pH measurements were recorded using an EDT Instruments RE 357 Tx meter.

The HPLC columns used were Synergy Polar-RP 4.6 mm × 75 mm 4 μm supplied by Phenomenex and Primesep B 4.6 mm × 150 mm 5 μm, Primesep 200 3.2 mm × 150 mm 5 μm and Obelisc R 3.2 mm × 150 mm 5 μm supplied by SIELC Technologies.

2.3. Mobile phase preparation

0.1% aq. TFA (pH 1.9) was prepared by adding 1.0 mL TFA to 1000 mL water. 30% MeOH/70% ACN was prepared by adding 300 mL MeOH to 700 mL ACN. 1.36 g KH_2PO_4 was dissolved in 1000 mL water to give a 10 mmol L^{-1} (pH 4.5) solution. 0.1% aq. HCOOH pH 4.0 buffer was prepared by adding 1.0 mL HCOOH to 1000 mL water and adjusting the pH to 4.0 with 10 mol L^{-1} NaOH solution.

2.4. Sample preparation

The single sample solutions of components **1**, **2**, **3**, **5** and **6**, used in Fig. 2 were all prepared at a concentration of 1.0 mg mL^{-1} in ACN. For Figs. 3–5, two mixed solutions were prepared with the individual component concentrations consistent with the reaction mixture. Mix 1 contained components **1**, **2** and **3** at 0.40, 0.50 and 1.50 mg mL^{-1} in ACN, respectively whilst mix 2 contained components **4**, **5** and **6** at 0.18, 0.12, and 0.16 mg mL^{-1} in ACN, respectively.

2.5. Chromatographic method

The chromatographic methods used are summarised in the figure legends. The detector wavelength monitored was 300 nm in all cases. Where composite injections were made from two vials, the Agilent 1100 injection program was used to aspirate, mix and inject the samples.

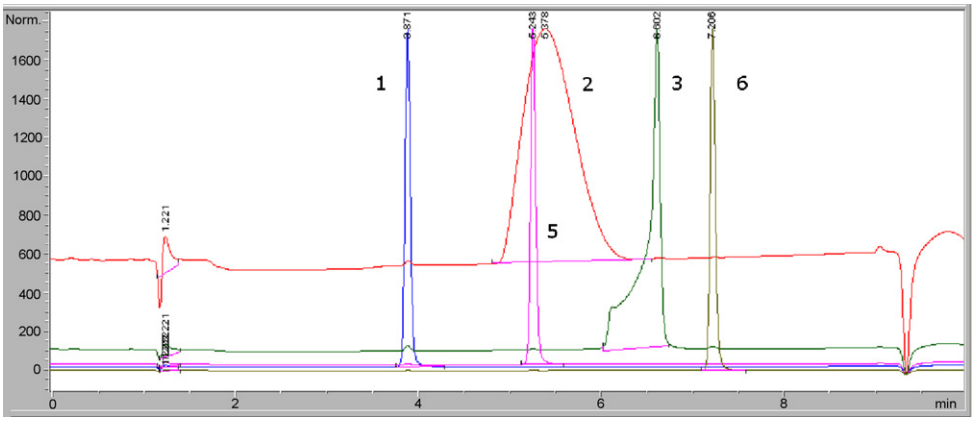

Fig. 2. Chromatographs of compounds **1**, **2**, **3**, **5** and **6**. Column: Synergy Polar-RP 4.6 mm × 75 mm 4 μm. Mobile phase: A was 0.1% aq. TFA; B was ACN. Flow rate 1.0 mL min^{-1}. Initial 90% A 10% B. Linear gradient 0–8.0 min, 90% B; 8.0–8.2 min, 10% B. Run time 10.0 min. T = 60 °C. UV detection at 300 nm. Injection: 2 μL.

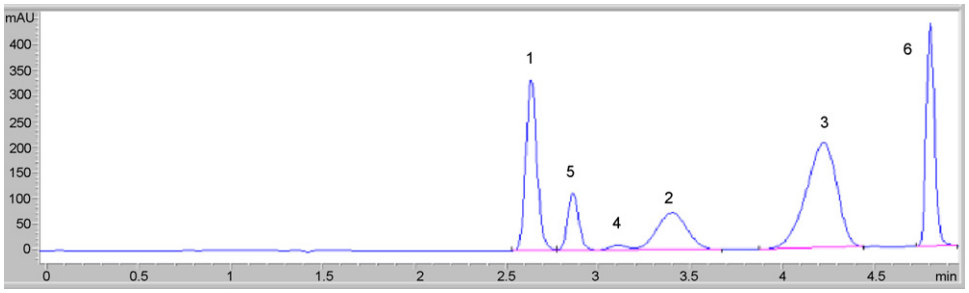

Fig. 3. Chromatograph of simulated reaction mixture. Column: Primesep B 4.6 mm × 150 mm 5 μm. Mobile phase: A was 0.1% aq. TFA; B was MeOH. Flow rate 1.5 mL min^{-1}. Initial 70% A 30% B. Linear gradient 0–2.5 min, 57% B; 2.5–3.5 min, 95% B; 3.5–3.6 min, 30% B. Run time 5.0 min. T = 55 °C. UV detection at 300 nm. Injection: 2 μL mix 1 + 2 μL mix 2 performed using Agilent 1100 injection program.

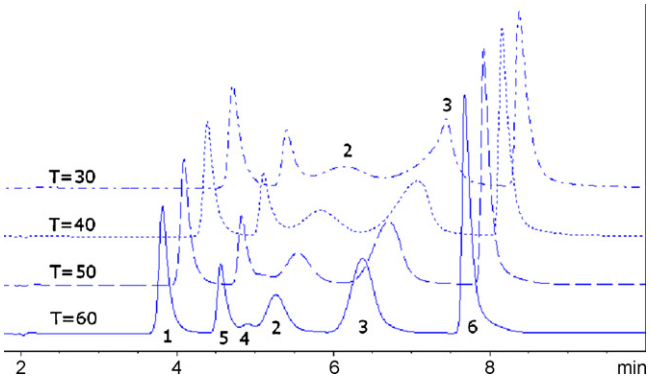

Fig. 4. Temperature study on simulated reaction mixture. Column: Primesep B 4.6 mm × 150 mm 5 μm. Mobile phase: A was 0.1% aq. TFA; B was 30% MeOH/70% ACN. Flow rate 1.0 mL min^{-1}. Initial 80% A 20% B. Linear gradient 0–8.0 min, 85% B; 8.0–8.2 min, 20% B. Run time 10.0 min. T = 60, 50, 40, 30 °C. UV detection at 300 nm. Injection: 2 μL mix 1 + 2 μL mix 2 performed using Agilent 1100 injection program.

3. Results and discussion

3.1. Column evaluation

Our initial results (data not shown) confirmed that conventional HPLC methods using 0.1% aq. TFA/ACN linear gradients and C8 or C18 reversed phases gave very poor peak shapes for the β-diketone compounds **2** and **3**, even at an elevated column temperature of 60 °C. However, improved results were obtained with a few modified phases. Typical of these was the Synergy Polar-RP column, Fig. 2, where the broadness of the peak for compound **2** and the unsymmetrical nature of the peak for **3** can be compared to the sharp symmetrical peaks of the non-diketone compounds **1**, **5** and **6**.

Subsequent evaluation of a series of mixed-mode columns, Primesep B (reversed-phase strong anion exchange), Primesep 200 (reversed-phase strong cation exchange) and Obelisc R (reversed-phase cation and anion exchange) using a simple 90% 0.1% aq. TFA or 10 mmol L^{-1} KH_2PO_4 (pH 4.5)/10% ACN to 10%/90% linear gradient over 8.0 min with a 2 min re-equilibration (flow rate 1.0 mL min^{-1} for Primesep B, 0.5 mL min^{-1} for Primesep 200 and Obelisc R) all produced sharp and symmetrical peak shape for the β-diketone compounds (**2** and **3**), whether they possessed cationic or anionic exchange characteristics or both. In fact, other SIELC phases such as Primesep B2 and Primesep D also gave analogous peak shapes. This suggests that a simple retention mechanism involving only a combination of ion exchange and reversed phase characteristics is unlikely to explain the observed chromatographic behaviour and would warrant a further in-depth study. Further information about the known separation mechanisms on these types of phases can be found at http://www.sielc.com.

3.2. Method development and temperature evaluation

Addition of the third β-diketone compound (**4**) into the above evaluation showed complete lack of resolution between it and the non-diketone impurity (**5**). In our laboratory, a standard procedure to investigate this type of problem is to switch the organic phase to both MeOH and a series of MeOH/ACN mixes. This process resulted in an optimised method using a 0.1% aq. TFA/MeOH linear gradient on a 150 mm × 4.6 mm, 5 μm Primesep B column capable of separating all 6 components within the required 5 min (Fig. 3).

As a part of this process, a temperature study was carried out on an intermediate method in an attempt to reduce the column temperature from 60 °C closer to the manufacturer's recommendation of 40 °C. The results are shown in Fig. 4. 30% MeOH in the ACN organic mobile phase was the minimum needed to give a usable separation of all six compounds at 60 °C. However, a stepwise reduction in temperature from 60 °C to 30 °C resulted in complete loss of peak shape and resolution of the β-diketone components **2**, **3** and **4**. Changing to a 100% MeOH organic mobile phase regained a little peak shape allowing the final method in Fig. 3 to be run at 55 °C. The requirement for MeOH to achieve the desired separation restricted the choice of column used to the Primesep B phase which does not contain acidic functional groups which would esterify under these conditions. The Primesep D phase could also be used but showed no advantage.

This temperature dependence of the peak shape for the β-diketone compounds was only an issue when TFA was used in the aqueous phase. Evidence is given in the following section for methods using a lower column temperature.

3.3. Counterion and pH evaluation

A general observation from the extensive method development carried out was that the peak broadening observed for the β-diketone components in the final method shown in Fig. 3 and its temperature dependence was only associated with the use of TFA. In addition, the resolution of peaks 4 and 5 was conditional on the use of both TFA and MeOH. However, better peak shapes were generally obtained for the β-diketone components using a variety of counterions, such as phosphate, sulphate, methanesulphonate, oxalate and perchlorate over the recommended pH range of the column (pH 1.9–4.5 for Primesep B). Although the t_Rs of the β-diketone components were relatively insensitive to these changes, the t_R of the phenol reactant (Fig. 1 compound **1**) proved very sensitive to changes in pH. An increase in t_R was seen as pH increased, often resulting in a change of elution order. The calculated pK_a 7.2 (ACD/Labs 11.00) would suggest that the phenol is un-ionised throughout this pH range and that it is unlikely that an ion exchange mechanism can explain this behaviour.

An example of these findings is given in Fig. 5 where a 0.1% aq. HCOOH pH 4.0/MeOH eluent system gives excellent peak shape, for

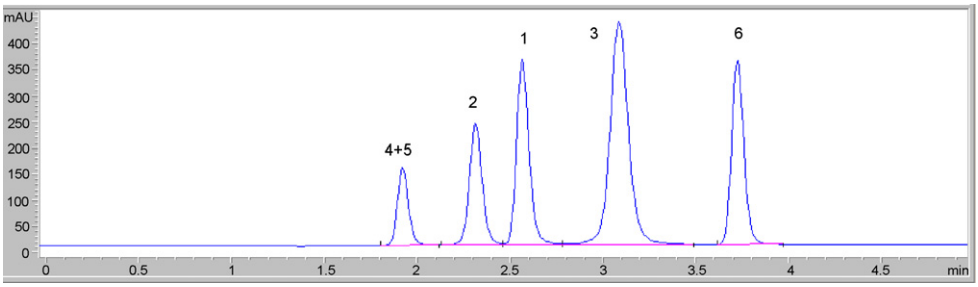

Fig. 5. Chromatograph of simulated reaction mixture. Column: Primesep B 4.6 mm × 150 mm 5 μm. Mobile phase: A was 0.1% aq. HCOOH pH 4.0; B was MeOH. Flow rate 1.5 mL min^{-1}. Initial 50% A 50% B. Linear gradient 0–3.8 min, 80% B; 3.8–3.9 min, 50% B. Run time 5.0 min. T = 40 °C. UV detection at 300 nm. Injection: 2 μL mix 1 + 2 μL mix 2 performed using Agilent 1100 injection program.

all components, at 40 °C with increased retention of the phenol but complete loss in resolution of peaks 4 and 5.

3.4. Final method reproducibility

The reproducibility of the final method described in Fig. 3 was confirmed by performing 30 consecutive injections of the simulated reaction mixture. The RSDs of the t_Rs and peak areas for the three β-diketone components **2**, **3** and **4** were 0.07, 6.0; 0.07, 1.6 and 0.06, 8.8, respectively.

4. Conclusion

The primary objective of this work was to develop a rapid HPLC method for the analysis of reaction mixtures containing the six components shown in Fig. 1. This was successful in that the chromatogram shown in Fig. 3 was obtained with good reproducibility for the t_Rs and peak areas for the three β-diketone components **2**, **3** and **4**.

The second objective of this work was to demonstrate that it is possible to solve a difficult peak shape/resolution problem by changing to modern stationary phases. These results show that it is possible to obtain good peak shapes and resolution for β-diketone compounds by direct sample injection, with no sample pre-treatment, using simple conventional mobile phase systems when combined with the modern mixed-mode phases available from SIELC Technologies. These mobile phase systems can be ACN or MeOH based with a variety of aqueous phases consisting of counterions such as TFA, phosphate, sulphate, methanesulphonate, oxalate and perchlorate over the recommended pH range of the columns.

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