Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1079 (2005) 349–353
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Application of main component fraction collection method for purification of compound libraries

T. Karancsi *, L. Gödörházy, D. Szalay, B. Papp, L. Németh, F. Darvas

ComGenex Inc., Záhony u. 7, 1031 Budapest, Hungary

Available online 7 April 2005

Abstract

In order to support high-throughput library purification, a novel UV triggered fraction collection method was developed in which a maximum-seeking-algorithm-driven, six-port valve collects the largest chromatographic peak. This straightforward strategy achieves the one sample-one fraction approach, thus resulting in a simpler and less error prone workup procedure. The effectiveness of this main component fraction collection method will be illustrated here by the results of the purification of compound libraries (altogether 6086 compounds, having an averaged success rate of 79.4%). Advanced applications, where the desired component differs from the main component, will also be discussed.

Keywords: Purification; Fraction; Compound libraries

1. Introduction

The widespread application of large size compound libraries and high-throughput screening methods in drug research has created an immediate need for high throughput analytical techniques capable of characterizing the test compounds. In order to maintain the reliability of the screening results the compounds often must be purified prior to the test procedure. Due to their effectiveness, in most of the cases, preparative chromatographic techniques, like supercritical fluid chromatography or HPLC [1–4], are used for this purpose. The general process of chromatographic purification consists of three major parts: the separation of the sample components, development of a fraction collection strategy, and the post-purification processes (like fraction qualification, evaporation, weighing, and transfer to a proper vial format) (Fig. 1). In order to find an effective high throughput purification method for a library requires optimal combination of solutions for these three major task categories.

The first important issue is the application (or development) of a proper chromatographic method to separate the components of the sample from each other. Since, due to the large sample numbers, the time and usually the sample quantity is limited, a thorough individual method development is practically impossible. Therefore, usually fast universal gradient methods must be applied for the library members [5–11]. This approach can further be optimized using the primary analytical HPLC results as a basis in generation of a preparative scale method [12–14].

After the desired components are separated well from the impurities a proper fraction collection strategy should be defined. Due to the lack of pilot-runs, where the retention time of the expected compound is regarded unknown, the safest strategy is to collect every detected peak of the chromatogram. This method has the disadvantage of requiring collection of several fractions for one injected sample (multicomponent collection), which requires an oversized fraction collector system. Moreover, analytical capacity must be allocated for characterization of the fractions in excess [2,15]. To decrease the number of the collected fractions, the following methods can be applied: (1) collection parameters can be further specialized, such as using narrower retention time window around the desired component or higher intensity threshold based on the predictions from the analytical results [12–14], (2) detection wavelengths can be varied (especially for col-

* Corresponding author. Fax: +36 1 214 2310.
E-mail address: tamas.karancsi@comgenex.hu (T. Karancsi).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.02.079

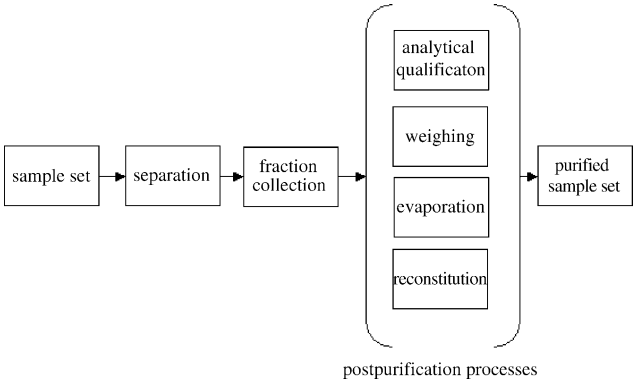

Fig. 1. The general overview of the purification process.

ored compounds). (3) A more informative detection method can be applied, such as mass spectrometry, for triggering the fraction collection [11,16–20].

After collecting the fractions the remaining tasks are summarized as post-purification processes (e.g. characterization of the collected fractions, culling with respect to the results, evaporation, weighing, reconstitution to the final vial). To make the overall purification process faster and more cost effective, it is necessary to reduce the number of the collected fractions per sample. The above solutions intended to decrease the number of fractions either risk losing the desired compound (limited accuracy of the retention time predictions), or require more expensive and more sophisticated hardware (MS). The main challenge in method development for high throughput library purification is to achieve an acceptable compromise among these factors.

Recently, we have published a straightforward new approach for fraction collection, which could be combined with the currently used triggering methods [21]. The main component fraction collection (MCFC) method uses a maximum-seeking-algorithm-driven, six-port collector valve to select the largest chromatographic peak within a retention time window defined in the chromatographic method. This data dependent collection method, capable of differentiating between the detected peaks by relative ordering, guarantees the collection of the main chromatographic component independently of its retention time or absolute intensity. Since application of this method provides one fraction per injection the essential capacity for post-purification processes can be decreased as well. Here, we present further examples for library purification as well as applications where the desired component differs from the chromatographic main component.

2. Experimental

Merck LaChrom low-pressure gradient HPLC system was applied for chromatographic separation, containing an L7150 pump, a D7000 interface module, and an L-7400 UV detector (Merck-Hitachi, Darmstadt, Germany), using Merck HSM software to control the data acquisition.

The HPLC experiments were performed on 5 micron 25 × 100 Purospher STAR RP-18 endcapped columns (Merck, Darmstadt, Germany). The ternary gradient was built up from 5% acetonitrile containing water (eluent A), acetonitrile (eluent B) and column washing solvent that is 70% acetonitrile: 30% isopropanol: 0.1% formic acid (eluent C). The most frequently used gradient program was the following: 0% B–100% B in 6 min, hold for 3 min, then turn to 100% C for 1 min, then 3 min equilibration at 0% B. The flow rate was 15 mL/min, the detection wavelength was 220 nm.

For the automated sample introduction and fraction handling we use Cavro 9651 liquid handler (Tecan Systems, San Jose, CA, USA) equipped with one arm, a 15 mL syringe pump, a high-pressure injector valve (C6UW) and a low-pressure collector valve (C22Z-3186, VICI AG, Schenkon, Switzerland). The liquid handler is furnished to handle 964 mL sample vials and 9625 mL fraction vials in one run.

MCFC is coordinated by an electronic board (developed in this laboratory), with an embedded programmable microcontroller (PIC16F877A, Microchip Technology, Chandler, AZ, USA). The microcontroller continuously monitors the detector signal within the retention time window (predefined in the chromatographic method using event output signals), recognizes the peaks and applies a maximum seeking algorithm with respect to their height. To provide enough time for this process, a delay loop with a volume of 18 mL is inserted between the detector and the collector valve. In case a peak is detected, a six-port collector valve turns to collect position (after a proper delay time) and the appropriate fraction will be directed to the collector loop (temporary buffer) having a volume of 15 mL, with capacity to collect a peak having a 1-min base peak-width. After a minute the collector valve turns back to stand-by. The following peak will be compared to the previous one and, if larger, the trapped fraction will be exchanged to the new one. Finally, at the end of the purification process, the content of the collector loop will be emptied to the appropriate fraction vial. Besides controlling the fraction collection, this unit functions as a master to synchronize the work of the different modules of the system.

The performance of the purification systems is monitored by using pure test compounds with a 85% or higher acceptance criteria for recovery (by weight). A more detailed technical description of the system can be found elsewhere [21].

3. Results

Below three different types of case studies are presented to illustrate the applicability of the MCFC method. The first example is the purification of seven compound libraries (Lib.A–Lib.G, altogether 6086 compounds). In these cases, the compound sets subjected to purification were se-

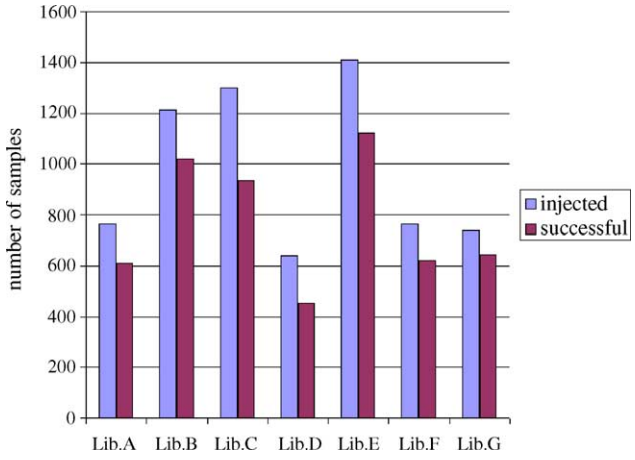

Fig. 2. Success ratios of the purification of 6086 samples from seven different libraries.

lected with respect to their primary QC results (measured by HPLC/MS technique using 220 nm detection wavelength). The initial purity was in the range of 40–89%, and the targeted purity was 90% or higher. The results of the process are summarized in Fig. 2, the success rates varying from 71 to 88% with an average of 79.4%. The majority of the unsuccessful purifications can be attributed to the poor chromatographic separations that are apparent in the analytical scale experiments as well.

4. Advanced retention window based application

In case of Lib.H and Lib.I we have encountered severe purification problems during the pilot production phase. Due to the incomplete reaction and the presence of side products in most of the cases the desired compound was only the second largest peak in the chromatogram. Since we managed to separate the main impurity (that is actually the chromatographic main component) far from the expected end product, the beginning of the collection window can safely be defined after the retention time of the non-desired major component and still well before the desired one. After exclusion of the non-desired chromatographic main component from the monitored chromatogram section (bracketed with dashed lines in Fig. 3), this general method was found to be safe enough for handling these compounds even without primary (pre-purification) analytical results. Within a library the same chromatographic method and the same collection window parameters were used. The overall success ratios were 87% for Lib.H and 73% for Lib.I (based on purification of 288 and 392 compounds, respectively). In this case,

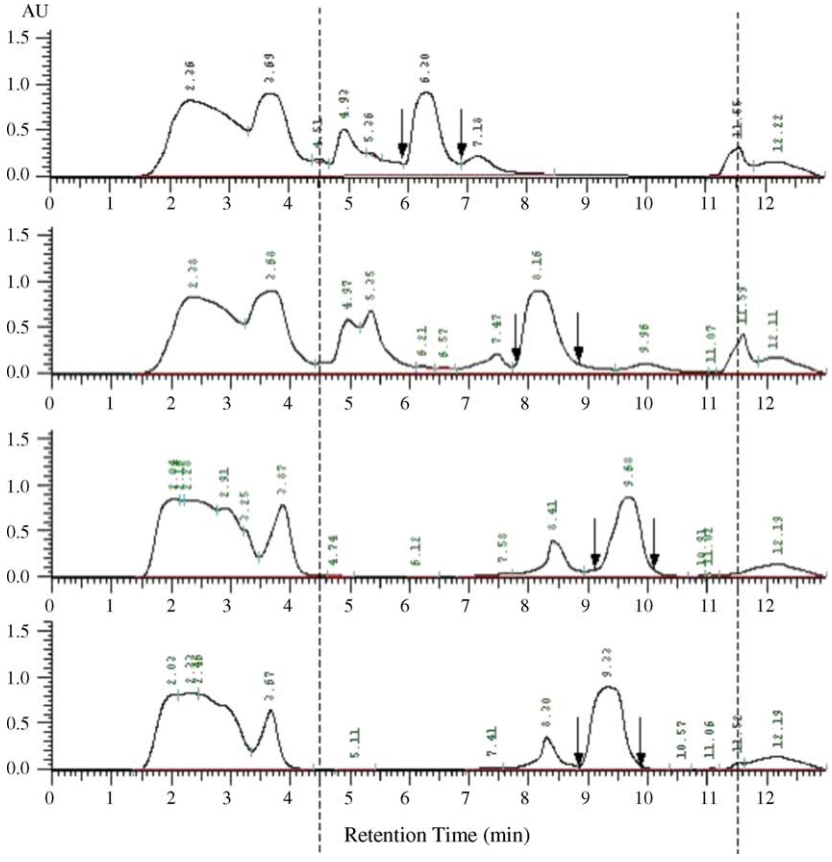

Fig. 3. Typical preparative chromatograms from the purification of Lib.H and Lib.I. The dashed lines bracket the time period when the main component fraction collection process is active. The arrows mark the finally collected chromatographic peaks.

the success ratios incorporates the failures in synthesis as well.

5. Wavelength selection based application

Another solution was adopted for the purification of Lib.K, where the crude products were rather complex mixtures according to the HPLC-MS experiments using 220 nm detection wavelength. In most of these cases the desired compound was only a minor component. Fortunately, the expected products have significant absorption properties having intense yellow color, so we built our purification strategy upon this specific feature of the library. Using 380 nm detection wavelength for the preparative runs, the risk of false collection was minimized, since the desired compound became the main component (in most cases the only detectable component). This method was able to distinguish a regioisomeric impurity, which has identical molecular weight, but different spectroscopic properties. As an example, two analytical HPLC/MS experiments are represented by color-coded chromatograms in Fig. 4., measured before and after the purification at 220 nm detection wavelength. As one can see in the middle trace (pre-purification QC) the sample contains two compounds (regioisomers) of the expected molecular weight, and the desired isomer does not correspond to the main chromatographic component (marked with an asterisk). The upper trace represents the product purity after the purification using 380 nm detection wavelength, and where the only detected component was the targeted isomer (Fig. 4. bottom trace). We achieved 78% success rate for this library on a 534-membered sample set.

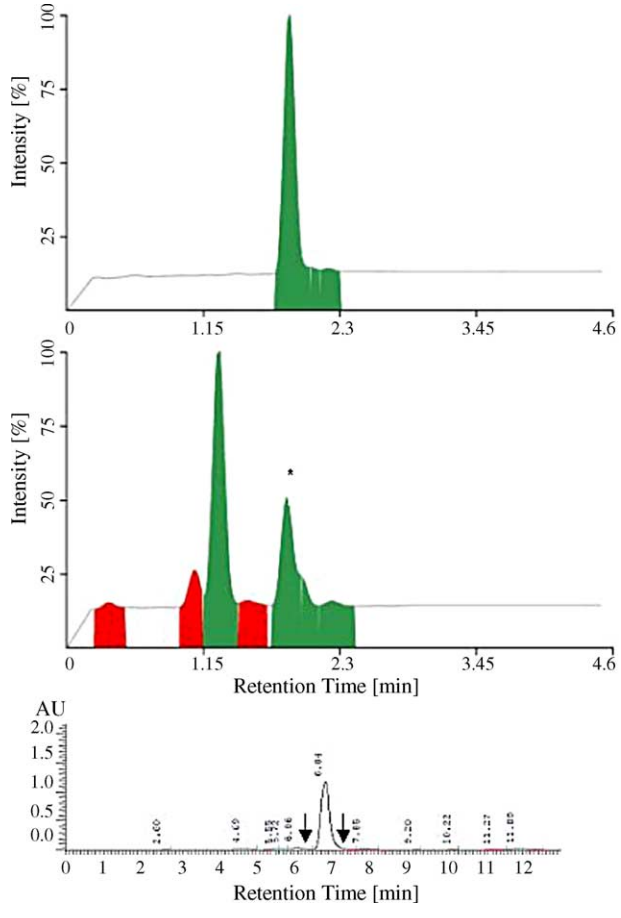

Fig. 4. The analytical chromatogram of a compound from Lib.K before (middle) and after (top) purification using 380 nm detection wavelength (bottom trace, the arrows mark the collection events). The desired isomer marked with an asterisk. The green color represents the fact that the compounds elute in those appropriate chromatographic peaks were detected as expected compounds by the mass spectrometric detector.

6. Discussion

Results presented in this paper demonstrate that the MCFC method can be successfully applied even in the case of rather complex samples in unattended manner. The main benefit of the MCFC method is the realization of the one-sample/one-fraction approach. In a purification process, where a conventional UV triggered collection method is applied, the number of the expected fractions (so the required number of the fraction vials) cannot be estimated accurately. Thus, the collector capacity should be overestimated, which can result in multiplication of fraction collectors as well. Using specialized collection parameters such as higher intensity thresholds or narrow retention time windows around the desired peaks to decrease the number of fractions, one achieves limited success without prior optimization of the preparative separation for unattended library purification. The situation is better if the results of the analytical experiments are available to be used for preparative scale method development. An improvement of the separation can be achieved by automatic generation (or selection) of focused gradient programs for preparative chromatography based on pre-purification analytical data. For method scale-up a well-defined relationship is required between the analytical and preparative chromatographic parameters (usually the same stationary phase and the same eluent system is recommended). Otherwise the desired component may be lost due to the unreliability of the predictions.

A widespread solution to decrease the fraction number is the application of a more informative detection method for triggering such as mass spectrometer. The MS-triggered purification, besides distinguishing components having the desired molecular weight, provides possibility to separate components effectively even if they are not separated completely chromatographically (using mass spectral purity as a collection parameter). However, for samples containing impurities having the same molecular mass as the desired compound (regioisomers) this technique results in multiple fractions as well.

If the final fraction number is unpredictable, a post-purification culling process is required during which each fraction is qualified by analytical measurements with respect

to their purity and identity. Based on the results, the proper fractions should be selected and transferred to their final storage vials. The more general the collection method the more analytical capacity has to be allocated for this process.

By contrast, the application of the MCFC method always results in one fraction, therefore only a final analytical run is required to close the QC data file. Additionally, the fraction vial–sample vial relationship remains straightforward during the whole process, since the fraction plate can be regarded as a purified daughter plate of the original sample plate.

The simple UV-triggered method requires a conventional preparative HPLC system equipped usually with oversized (or multiplied) fraction collection modules, as well as an excess of analytical capacity for the post-purification qualification of the fractions. Although the MS-triggered purification systems require less analytical runtime due to the decreased fraction number, an MS detector is necessary. The MCFC method requires only the electronic unit with the embedded intelligence for the online decision-making algorithm.

Obviously, the MCFC method has no positive effect on the separation efficiency, since that is determined by the applied chromatographic method. Moreover, due to the fixed-volume collection loop, if a desired component gives a half-min narrow peak just after an impurity having also a half-min peak, these two components will be collected together, although chromatographically separated. This fortunately unusual example highlights that the MCFC method requires some advance chromatographic method development prior to the purification run.

7. Conclusion

The cost effective MCFC method fits well with the present armory of library purification. Its application is ideal for samples contaminated with minor impurities, which are well separated from the desired chromatographic main component. Nevertheless, it can also be used for more complex samples, even if the desired compound is not the main component. Incorporating this simple hardware modification, this approach results in a significant decrease in analytical capacity required for qualification of the collected fractions.

References

[1] M. Ventura, W. Farrell, C. Aurigemma, K. Tivel, M. Greig, J. Wheatley, A. Yanovsky, K.E. Milgram, D. Dalesandro, R. DeGuyman, P. Tran, L. Nguyen, L. Chung, O. Gron, C.A. Koch, J. Chromatogr. A 1036 (2004) 7.
[2] D.B. Kassel, Chem. Rev. 101 (2001) 255.
[3] X. Cheng, J. Hochlowsky, Anal. Chem. 74 (2002) 2679.
[4] R.H. Griffey, H. An, L.L. Cummins, H.J. Gaus, B. Haly, R. Herrmann, P.D. Cook, Tetrahedron 54 (1998) 4067.
[5] W.C. Ripka, G. Barker, J. Krakover, Drug. Discov. Today 7 (2002) 56.
[6] R.A. Tommasi, L.W. Whaley, H.R. Marepalli, J. Comb. Chem. 2 (2000) 447.
[7] C. Edwards, D.J. Hunter, J. Comb. Chem. 5 (2003) 61.
[8] W. Leister, K. Strauss, D. Wisnovski, Z. Zhao, C. Lindsley, J. Comb. Chem. 5 (2003) 322.
[9] R. Xu, T. Wang, J. Isbell, Z. Cai, C. Sykes, A. Brailsford, D.B. Kassel, Anal. Chem. 74 (2002) 3055.
[10] J.P. Kiplinger, R.O. Cole, S. Robinson, E.J. Roskamp, R.S. Ware, H.J. O'Connell, A. Brailsford, J. Batt, Rapid Commun. Mass Spectrom. 12 (1998) 658.
[11] L. Zeng, D. Kassel, Anal. Chem. 70 (1998) 4380.
[12] B. Li, C.Y. Chan, J. Comb. Chem. 5 (2003) 834.
[13] K.F. Blom, R. Sparks, J. Doughty, J.G. Everlof, T. Haque, A.P. Combs, J. Comb. Chem. 5 (2003) 670.
[14] B. Yan, N. Collins, J. Wheatley, M. Irving, K. Leopold, C. Chan, A. Shornikov, L. Fang, A. Lee, M. Stock, J. Zhao, J. Comb. Chem. 6 (2004) 255.
[15] R. Richmond, E. Görlach, J. Seifert, J. Chromatogr. A 835 (1999) 29.
[16] G.R. Elridge, H.C. Vervoort, C.M. Lee, P.A. Cremin, C.T. Williams, S.M. Hart, M.G. Goering, M. O'Neil-Johnson, Lu Zeng, Anal. Chem. 74 (2002) 3963.
[17] M. Bauser, J. Chromatogr. Sci. 40 (2002) 292.
[18] L. Zeng, L. Burton, K. Yung, B. Shushan, D.B. Kassel, J. Chromatogr. A 794 (1998) 3.
[19] J. Isbell, R. Xu, Z. Cai, D.B. Kassel, J. Comb. Chem. 4 (2002) 600.
[20] U. Rosentreter, U. Huber, J. Comb. Chem. 6 (2004) 159.
[21] T. Karancsi, L. Gödörházy, D. Szalay, F. Darvas, J. Comb. Chem. 7 (2005) 58.