Applications of HPLC in Pharmaceutical Engineering

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Abstract: The pursuit of high quality drugs leads to increasing demand for technique with high performance in mixture separation. Pharmaceutical industry is a field requiring fine separation technology. High performance liquid chromatography (HPLC) is commonly seen in pharmaceutical study and manufacture in separation processes for diverse aims. In this paper, the related information from some databases are collected and summarized to discuss three major applications including the purification of raw material, quality analysis of drug product and assay of metabolites. Evidences show that HPLC has broad purpose by pharmaceutical engineering. This paper is intended to offer an overview on applications of HPLC in pharmaceutical engineering and provide ideas for improvements of technique with new methods.

1 INTRODUCTION

Chromatography has been developed a lot in the last hundred years since the initial separating the plant pigments. For the over decades, its analytical ability have been greatly improved by the advance of equipment. High performance liquid chromatography (HPLC) is a rapid separation instrument commonly used in pharmaceutical engineering, which is based on the principle that different materials own different distribution coefficients in a certain stationary phase and mobile phase.

Pharmaceutical engineering is a field has high requirement for separation. The fast running speed of HPLC maks it one of the most applied tools in substances separation and real-time detection in several important segments pharmaceutical industry. Connected with detective devices, it can help to detect residual substances, determine the drug content, analyse drug metabolism and so on (Ahuja 2017). HPLC plays an important role in the producing process of those products with high purity requirement. It is applied in the purification of alkaloid and peptide which takes a quite low proportion in the mixture (Wu et al. 2013). Besides, it is also widely applied in drug metabolism analysis for its high separation efficiency.

Recently, more and more specific HPLC methods are developed for the research on certain drug or preparation, which helps people to know better about the drug's properties and the improve its quality. Therefore, the following paragraphs will focus on three major applications of HPLC: purification, quality analysis and metabolism analysis.

2 RELEVANT ANALYSIS ON HPLC

2.1 HPLC for Drug Purification

A considerable part of the existing drugs come from natural products of plants or microorganism. Therefore, purification with high efficacy is an indispensable stage during drug discovery and manufacture. HPLC displays a good performance in separating and purifying substances.

One of the problems in deriving natural product is that the content of the active ingredient is very low and the components are complicated, making separation difficult. Fortunately, HPLC helps this process easier. Liao X set up a method to determine the active components of Osmanthus fragrans roots by HPLC-MS/MS (Liao et al. 2021). In this experiment, 36 compounds were detected in the separation and one of them was newly found in the work. Tandem mass spectrometer (MS/MS) helped to measure the relative molecular mass of each component segregated by HPLC, providing key information for chemical structures. Hong Y and his partners also used a similar method (HPLC-MS/MS)

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to determine the bioactive ingredients of Cercis chinensis Bunge fruits (Hong et al. 2020). Gallic acid was found to account for the largest proportion. This has not been reported before, indicating some new medicinal value of the fruits.

High throughput purification is demanded in pharmaceutical industry. However, it's not easy to realize that in the production of antibody, cytokine and other kinds of peptide drug. A large scale HPLC system used for purifying IgGs was validated by Schmitz S and his team (Schmitz et al. 2019). Three running pumps were paralleled and work simultaneously in this method to ensure the rapid removal of aggregates and endotoxins. They also used the autosampler to make the process automatic and enable continuous manufacturing.

Besides changing the parameter of chromatography, modifying the target product also makes purification efficient. Tag-assisted technology is quite useful in peptide drugs. The filler of column binds the corresponding groups that can specifically combine the tag of target protein. By this process, separation with high resolution can be realized by specific binding and elution. To simplify the procedures, the self-cleavable tags have been developed as well as the aggregating tags (Mmg et al. 2020). Such tags are intended to reduce the pretreatment or post processing.

High purity and high production are two goals that manufacturer wants to achieve. But these two objectives are often contradictory due to the limit of current manufacturing technique. Luca C. D. and her team have established a multicolumn continuous chromatography to solve the problem (Luca et al. 2020). Different from a classical forwardcurrent chromatography, the method employed countercurrent solvent to purify the products without a solid filler column. An automatic internal recycling was set in the system to increase production with acceptable purity.

2.2 HPLC for Drug Quality

Drug quality inspection is a necessary step to ensure the safety of preparations after production. Quality and impurity determination are two general test items for most preparations. HPLC is quite practical in preparation analysis due to high resolution and rapidity.

HPLC can be used for the determination of drugs with similar chemical structures when a suitable mobile phase and proper elution gradient are set. Trifluoperazine and prochlorperazine are both phenothiazine derivatives with only one different group on benzene ring. According to Dhabab (Dhabab et al. 2013), a determination method for these two drugs was conducted by using reverse phase HPLC (C18 column, acetonitrile as mobile phase, chlorperazine hydrochloride as internal standard), showing a good separation and acceptable accuracy in quality (recovery percent is higher than 96%).

By applying a chiral stationary phase, HPLC can be used to analyse the enantiomeric purity of certain drugs. Sertaline is an antidepression drug with two stereogenic centers. Ara B et al. have demonstrated a reverse phase method for determining enantiomeric purity of sertraline by using Chiralpak IG-3 column with acetonitrile-water-DEA mixtures (75:25:0.1, V/V/V) as mobile phase (Ara et al. 2020). The result showed that it could recognize another two stereoisomers of sertraline well within 15 min by a single-run approach, reducing the time consumption and expenditure of existing method.

HPLC is also applied in simultaneous estimation for compound preparations containing more than one active pharmaceutical ingredients. A simple, rapid HPLC method for estimation of three antivirus drugs (Lamivudine, Tenofovir, and Dolutegravir) is developed by Rao N M et al (Rao et al. 2015). They adopt a Inertsil ODS-3V C18 column with mobile phase of mobile preparation A (mixture of potassium dihydrogen orthophosphate buffer and methanol) and B (mixture of orthophosphoric acid and acetonitrile). These drugs can be separated within 14 mins and high mean percent of recovery by different added amount (all are over 99%), displaying a good applicability of this method.

Test of drug degradation is a necessary step to ensure its quality. HPLC can be applied to analyse the degradation substances of the drug under certain stress. Bisht R et al. have developed a reverse phase HPLC method connected with UV detector for determining the degradation of connexin43 mimetic peptide (Bisht et al. 2017). It presented good linearity between the concentration of 0.9-250 μg. The result showed that the peptide, which helps to treat inflammation, was sensitive to temperature and pH.

In addition, the analysis of excipient takes advantage of some detection technology combined with HPLC. Liposome is a good coating to improve drug's hydrophilicity. But one technical difficulty is how to determine the uniformity of each liposme as well as the drug inside it. Langer C and R Süss have created an HPLC method applied in a range of liposome drugs using diode array detector (DAD) for qualitative detection and corona charged aerosol detector (CAD) for quantitative detection (Langer et

al. 2021). The benefit of CAD is the non-reliance on the structures of substance. Instead, it depends on the number of charged atomized particles. Therefore, it can detect both the drug and liposomes after they were separated by HPLC. The method has been also validated for cyclodextrin coated drugs.

2.3 HPLC for Drug Metabolism

Drug metabolism analysis is an essential part during the study of pharmacokinetics. One of the common features of biological samples is the complex composition. So the technique for detection in animal experiment and clinical trial needs to be precise and effective. HPLC plays an important role in this part.

Plasma is the most commonly used biological sample. Sws A et al. have established an HPLC method combined with fluorescence detector (FLD) for determination of the metabolism of alpelisib in rat plasma (Sws et al. 2020). FLD is suitable for molecules which can emit fluorescence under certain wavelength radiation. Alpelisib, an antibreastcancer drug, has two aromatic rings which allow it to have a significant response in test. The result presented that the drug kept stable in blood for 24 hours.

Prodrug is an effective way to improve the bioavailability. Finding out the metabolic pathway is a significant step to ensure the safety of the drug. Nobilis M and his team developed a method for determination of the metabolites of new nabumetone (a type of anti-inflammatory prodrugs) (Nobilis et al. 2013). They first used liquid-liquid-extraciton to collect the compounds in liver microsomal fractions (including human tissue and rat tissue), and then employed HPLC with photo-diode array and tandem mass spectrometer to analyse the metabolites. 3-hydroxy nabumetone was found and it was inferred to be the first metabolite of new nabumetone after a further study on its biotransformation.

In order to make the detection comprehensive, radioactive elements are used to track the distribution and metabolism of drugs in the body. Accordingly, the radio-HPLC is established for this kind of test. Gaudin A et al. have developed a method to detect the squalenoyl adenosine nanoparticles (radio-labeled with 3H and 14C) in mice (Gaudin et al. 2015). In this paper, the nucleoside is covalented to squalene to extend its half-life. Mice plasma was used in the stability determination of the drug in vitro and it was separated by the HPLC and then detected by a radioactive element detector. The result indicated that the prodrug was able to persist for 1 hour and mainly absorbed by the liver and spleen.

Multidimensional chromatography is a technology that combines columns with different selectivity or several types of chromatography methods to improve the separation effect as much as possible. Roberta K et al. have established a method for the determination of albendazole metabolites in microsomal fractions of mice liver (Kátia et al. 2013). A chiral 2D-HPLC was employed in this work where a bovine serum albumin column was for sample clean-up and the other column for chiral resolution. Result showed that sulfonylation to albendazole occurred in liver tissue.

3 THE RESEARCH SITUATION OF HPLC IN PHARMACEUTICAL FIELD

Published papers in this field reflects the development of the technqiue. By using the visualized analysis of the database CNKI, the author input key words “HPLC drug” and derived the overall trend of the applications of HPLC in pharmaceutical field in China of recent years.

Figure 1: The annual published trend versus year

Figure 1 presents the change of the number relevant published papers. A huge growth of the number for over 1000 is seen from 2016 to 2017. Then the quantity has dropped a bit in the following

years but has increased again since 2020. This indicates that more attention has been paid to the study of HPLC in the field since 2016.

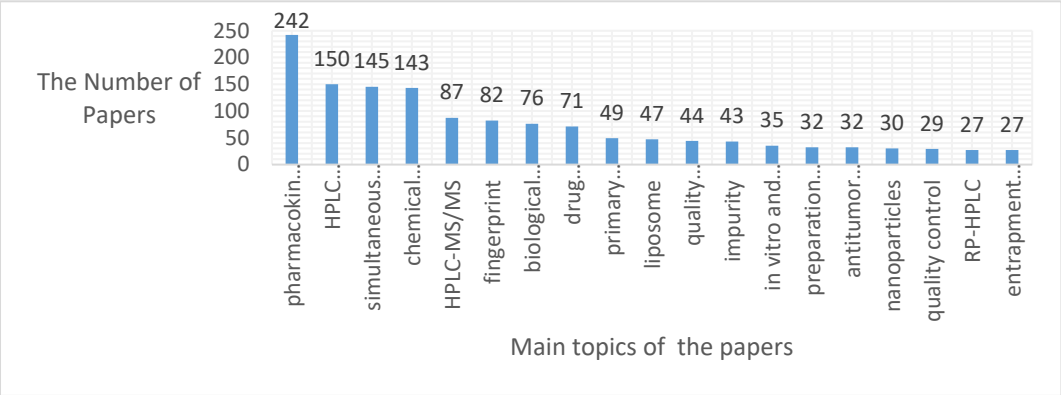

Figure 2: The distribution of major topics of the papers.

Figure 2 shows the different topics of the relevant papers and their respective quantities. Study on pharmacokinetics has taken up the highest number with 242 articles in the recent 5 years, while the HPLC method study ranked the second place with close numbers of simultaneous determination and chemical components. It can be inferred that HPLC mainly serves in medicine analysis and the study on purification for manufacture is rare.

Table 1: Application of IC-FLD/UV for the determination of different drugs.

Drugs	Sample preparation method	Analytical Technique Used	LOD	LOQ	Recovery
Bisphosphonates(etidronate,clodronate,amidronate, and alendronate)	Simple extraction	IC-FLD	50–100 µg/ mL	-	97.12-102.92%
Seven Stibonic Acids	-	IC-PDA and ESI-MS	0.3 µg/L	0.999 µg/L	-
Affffeine, theobromine, theophyllin	Simple extraction	IC-UV	0.03– 0.2 µg/mL	0.099–0.66 µg/mL	87–103%.
norfloxacin, ciprofloxacin, enoxacin	Direct filtration and injection	IC-FLD	50–105 µg/L	0.166–0.34 mg/L	100–104%
Zoledronic acid	Simple extraction	IC-UV	0.200– 1.200 mg /mL	-	-

Researches also concern about the comparison of the application of HPLC by different drugs. Ion chromatography (a type of HPLC with ion exchange resin as solid phase) is used to analyse ionized substance and table 1 gives figures of the determination of several drugs applying IC (Separovic et al. 2018). As it's shown, FLD and UV are commonly used as detectors, though the specific condition of the chromatography could vary a lot. The following data of each drug presented different ranges of LOD and LOQ, indicating that the performance of IC might be not universally referential, while the same thing happened by recovery with fluctuation of different degree. It might be even doubtful whether HPLC is suitable for a certain drug. Hence, a precise standard of HPLC applicability for drugs should be defined to provide more reference for quality analysis.

4 CONCLUSIONS

Overall, HPLC possesses good separation efficiency, universal applications and easy operation, thus making it practical in many fields of pharmaceutical engineering. However, some disadvantages of HPLC should not be neglected. Measurement uncertainty of the equipment is a factor influencing the quality of drug products. According to a study on HPLC determination of amoxicillin tablets (Muhammad et al. 2021), it has been found that the uncertainty of the whole process exceeded the recommended value and this has forced the manufacturer to take a higher risk. This suggests that addictied measures should be taken to check the accuracy during the process. Besides, environmental burden and human health must be considered since the organic solvents have a long-term effect by accumulation. Recently, more and more modified method using green solvents like

ethanol and acetic acid or even free solvent are being proposed (Mya et al. 2020, Mikhail et al. 2021). It is expected that more green and efficient methods will be found and established.

Although some problems exist in the HPLC method for pharmaceutical engineering, it still remains the necessary technique in substance purification, drug quality analysis and metabolism assay. Wide range of application including chemical drug, peptide drug, chiral drug and so on, makes it adopted nearly throughout the whole process. Overall, it is anticipated that more improved methods will be put forward to overcome the shortcomings. The findings above is aimed to provide some basic facts of the applications of HPLC in pharmaceutical engineering and more innovation of the method is expected to be inspired.

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