Development on in -situ flow measurement technology of high performance liquid chromatography

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Abstract

The infusion system (referred to pump) was the heart of High performance liquid chromatography (HPLC), which provided the power of HPLC. The critical parameter of the pump were the accuracy and stability of flow, which were directly related to the consistency and effectiveness of HPLC results. At present, the technical standards of HPLC are mainly JJG705-2014 and EDQM-OMCL, which were that Verification Regulation of Liquid Chromatograph and Quality Control Laboratory of European Medical Administration for flow of HPLC respective. In this study, a new portable HPLC flowmeter (PM-210plus) for HPLC was used. It adopted the volumetric principle, including the fully closed volumetric parts and the special constant force in situ detection structure, which realized in-situ measurement of HPLC flow and data traceability. The flowmeter was calibrated by Dynamic mass method water flow standard device of National Institute of metrology, China (NIM).Parameters of flow in-situ measurement were designed, including value error, stability, and temperature influence test. After the optimization of conditions, the flow range could be covered from 0.01 mL/min to 5.0 mL/min in situ conditions, the maximum allowable error was within ±1%, the stability was within 0.5%, and each measurement was completed in 3min. It fully met the requirements of JJG705 field flow measurement, and greatly improved the field measurement efficiency.

1. Introduction

High performance liquid chromatography (HPLC) method was a classical analytical technique with high separation efficiency, short analysis time and high sensitivity[1]. In the field of medicine research and development (R & D) and quality control(QC), HPLC methods were widely used in drug identification, impurity control, content determination and other aspects of drug inspection[2]. At present, HPLC methods have become the most important detection method for drug safety and effectiveness control, and widely included in pharmacopoeias of various countries. For example, the varieties of drugs involved in HPLC methods in the Chinese Pharmacopoeia (2020) accounted for about 70% of the total number with a total of about 6000[3]

The performance of HPLC directly affected the accuracy of drug results[4]. Therefore, HPLC have always been main object in the field of pharmaceutical supervision and certification. In China, JJG 705-2014[5] Verification regulation of liquid chromatograph is main standard for HPLC. In European Union(EU), EDQM-OMCL were the main documents[6], which were that Verification Regulation of Liquid Chromatograph and Quality Control Laboratory of European Medical Administration for flow of HPLC[7-8].

HPLC was consisted of four parts: infusion, separation, detection, and data processing. The infusion system (referred to as the pump) was the heart of HPLC, which provided the power of HPLC. The critical parameter of the pump was the flow, and the accuracy and stability of flow were directly related to the consistency and effectiveness of HPLC results[9-10]. With ultra-high performance liquid chromatograph(UPLC) and ultra-high performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) emerging, the demand for in situ flow measurement of UPLC have put forward[11-12], which required that the flow was measured in the pump working state of UPLC. Because flow of UPLC pump was set below 0.2 mL/min, and the method of manual weighing needed thirty mins each measurement and did not reflect the real working state of the pump[13-15].

The method were measured the flow with manual weighing and calculation by converting weight into volume through medium density. This method did not realize the demand of in-situ flow of HPLC. Most flow meter function also cannot measure the flow in situ in the market. In this study, a new portable HPLC flowmeter (PM-210plus) was used. It adopted the volumetric principle, including the fully closed volumetric parts and the special constant force in situ detection structure, which

realized in-situ measurement of flow and data traceability. The flowmeter was calibrated by Dynamic mass method water flow standard device of National Institute of metrology, China (NIM).

Parameters of flow in-situ measurement were designed, including value error, linearity, stability, and temperature influence test. Water and methanol as the medium were respectively used. After the optimization of conditions, the flow range could be covered from 0.01 mL/min to 5.0 mL/min in situ measurement conditions, the maximum allowable error was within ±1%, the stability was within 0.5%, and each flow was completed in 3min. It fully met the requirements of JJG705 field flow measurement. Through this study, the flow meter was used to replace the manual flow measurement of HPLC, which not only met the requirements of audit and tracking in the pharmaceutical industry, but also solved the problem of small flow in-situ field measurement of HPLC / UPLC[16].

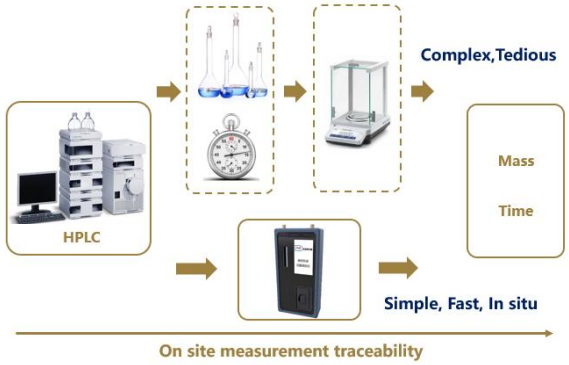

Figure 1: the HPLC flowmeter application and traceability

2. Material and method

2.1 Instrumentation and reagents

The instruments were Agilent 1200 HPLC system consisting of two LC pump and Agilent G1323B off-line plunger pump controller. (Agilent Corp, Germany). The electronic balance was AG285 from Mettler Toledo Company (Mettler Co., Ltd, Switzerland). PM-210plus HPLC Flowmeter was from Nanjing proficiency scientific instruments company (Proficiency company, China). HPLC grade methanol was purchased from Merck Company (Germany), the ultra-water was purified using a Milli-Q water purification system(18.2MΩ•cm).

2.2 Connection of HPLC flowmeter

As shown in the Figure 2, through using 1/16 peek connector, the fully closed measurement of HPLC flow was realized, which effectively reduced the error caused by the problem of liquid volatilization in the process.

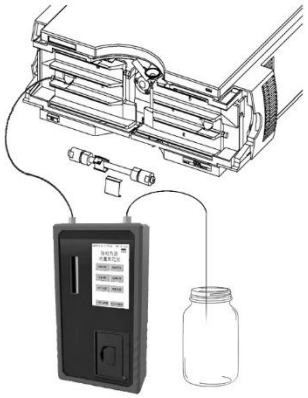

Figure 2: Connection diagram with the flowmeter and HPLC

2.3 Flowmeter for HPLC performance verification test

A stable and calibrated Agilent 1200 HPLC was selected as the flow generation source. In the working state, different test conditions were selected to evaluate and verify the indicators of the flowmeter.

2.3.1Performance test of HPLC flowmeter

Under the working conditions, different ambient temperature of 15℃、20℃、25℃、30℃, different solvent media selected as mobile phase, the flow of HPLC were measured by the flowmeter.

2.3.2 Stability test

Make statistics on the measurement results of the flowmeter within two hours and twelve months respectively.

3. Result and discussion

3.1 Indication error of HPLC flowmeter

Dynamic mass method water flow standard device (No.RGII2021-16574) of National Institute of metrology, China (NIM) was used to evaluate the indication error and repeatability. The results showed that the flowmeter could far enough meet the flow index of JJG705-2014.

Table 1： Indication error of common flow points for HPLC with the flowmeter

Flow setting value	Value of the flowmeter	Value of the standard device	Indication error	Repeatability	JJG705 Specified Indication error
2.0	1.9860	1.9795	0.33%	0.13%	±2%
	1.9871	1.9793	0.39%		
	1.9791	1.9744	0.24%		
1.0	1.0014	1.0022	-0.07%	0.12%	±3%
	1.0014	1.0023	-0.08%		
	1.0014	1.0037	-0.22%		

0.5	0.4937	0.4938	-0.01%	0.30%	±5%
	0.4883	0.4899	-0.31%		
	0.5002	0.5018	-0.30%		
0.2	0.1926	0.1932	-0.29%	0.09%	±5%
	0.1927	0.1935	-0.39%		
	0.1950	0.1955	-0.24%		

3.2 Performance test of HPLC flowmeter

3. 2.1 Influence of ambient temperature

In different ambient temperature of 15℃、20℃、25℃、30℃,respectively, the flowmeter was used to measure the flow of HPLC at setting value of 1.0mL/min. The results indicated that there was no difference in in the process of flow, and HPLC flowmeter met the
ambiental temperature requirements of JJG705-2014 and EDQM-OMCL.

Table 2: the results of flow in different ambient temperature

Ambient temperature °C	15	20	25	30
Setting value 1.0 mL/min	1.0023	1.0035	1.0042	1.0048
Relative error	0.23%	0.35%	0.42%	0.48%
Allowable error	±1%			

3.2.2 Media suitability test of mobile phase

Water, methanol, acetonitrile and isopropanol were selected as the media, and the flowmeter was used to measure the flow. The results showed that there was no obvious difference, indicating that the flowmeter could be applied to the flow measurement of common liquid chromatographic mobile phase media.

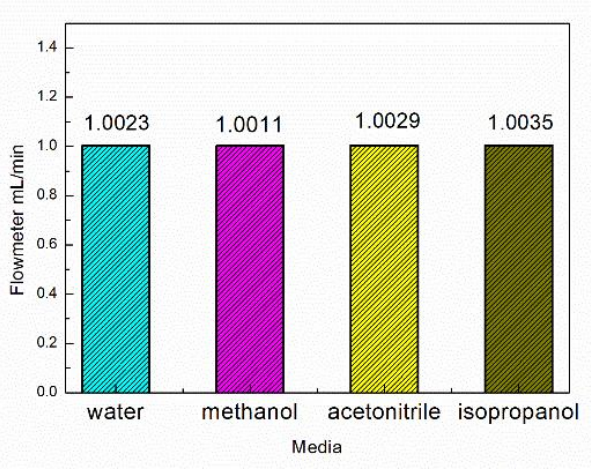

Figure 3: flow result comparison for different media test

3.3 Stability test

Under normal working conditions, the flowmeter were used for testing flow at various time. According to formula (1), the data of flow was calculated and short-term stability in two hours was less than 0.3%, and there was no abnormal value. During the 12-month time period, the flow were also tested and counted, and the results of flow were stable and the fluctuation ranged from 1.001mL/min to 1.006mL/min. The long-term stability in 12-month was 0.5%, so the results indicated that the flowmeter fully met the on-site measurement requirements of HPLC flow.

$$\Delta S = \frac{F_{max} - F_{min0}}{F_0} \quad (1)$$

ΔS: test stability

F_{max}: maximum in all test value

F_{min}: minimum in all test value

F_0: test initial value

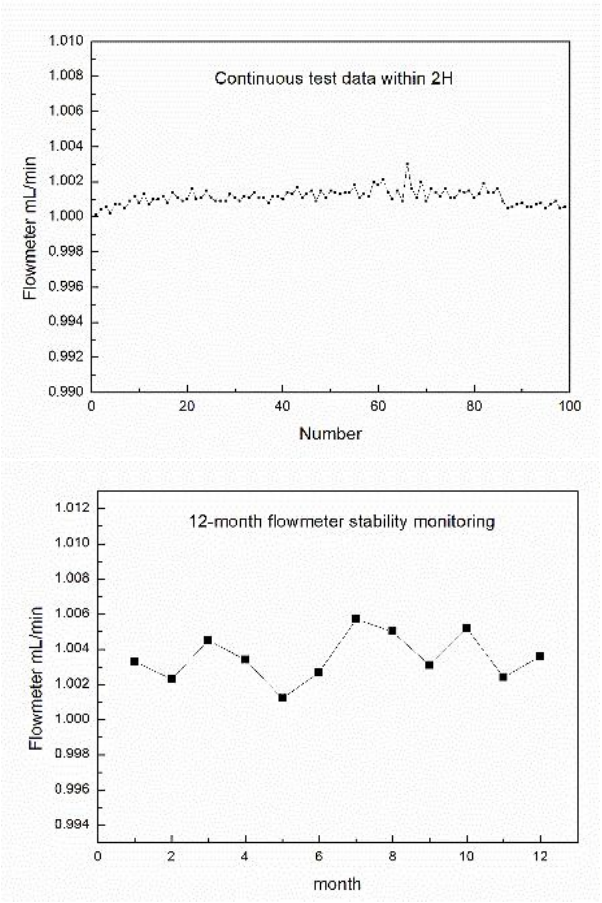

Figure 4: stability for the HPLC flowmeter

3.4 Comparison of time with manual test and HPLC flowmeter

Recorded the time of manual flow test and the flowmeter respectively, under different flow rates (0.5,1.0,2.0mL/min). The statistics were shown in figure 5, which showed that the test time was shortened, and reduced the risk of abnormal data caused by manual operation.

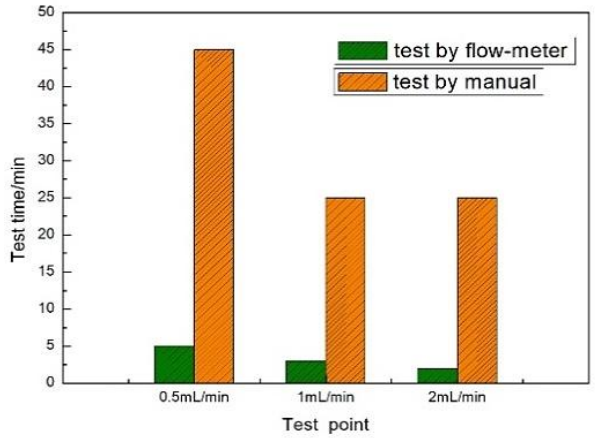

Figure 5: Comparison of time with manual test and HPLC flowmeter

4. Conclusions

A new type of flowmeter was designed and optimized based on various technical specifications such as JJG705 and Chinese Pharmacopoeia (2020). This flowmeter adopted the volumetric principle, including the fully closed volumetric parts and the special constant force in situ detection structure, which realizes in-situ measurement of HPLC flow and data traceability. The flow indication error test, temperature influence test and stability test were carried out to verify. The results showed that the flowmeter range can cover 0.01mL/min~5mL/min, the maximum allowable error was within ± 1%, and the stability was within 0.5%. The flow of common mobile phase media, such as water and methanol, were measured by the flowmeter. It fully met the on-site measurement of flow of HPLC and UPLC. The flowmeter was finally traceable through Dynamic mass method water flow standard device (No.RGII2021 -16574) of National Institute of metrology, China (NIM). Through this study, the automatic flowmeter will be used to replace the manual flow measurement of HPLC in the future. Which can solve the traceability problem of the small flow field measurement of HPLC.

References

[1] Xie M F, How to establish a HPLC Method for determination of related substances in drugs [J].*Chinese Journal of Pharmaceuticals,* **(01)**, 45-48, 2007.
[2] Wren S A C, Tchelitcheff P, Use of ultra-performance liquid chromatography in pharmaceutical development[J]. *Journal of Chromatography A*, **1119(1-2)**, 140-146, 2006.
[3] *National Pharmacopoeia Committee Chinese Pharmacopoeia* [M] (Beijing, China Medical Science and Technology Press), 2020.
[4] Li F, Li Y, Xu Z Y, Inportance of chromatographic system suitability test in analytical method setup[J], *Chinese Journal of New Drugs*,**28(06)**, 666-669,2019.
[5] National physical and Chemical Metrology Technical Committee: *JJG 705-2014 verification regulation of liquid chromatograph* [S], 2014
[6] The United States Pharmacopeial Convention USP**(1058)**
[7] OMCL Network of the council of Europe, *Quality Management Document PA/PH/OMCL (11)04-Annex1*[EB/OL].
[8] Shabir G A, Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization[J]. *Journal of chromatography A*, **987(1-2)**, 57-66, 2003.
[9] Masson P. Quality control techniques for routine analysis with liquid chromatography in laboratories[J]. *Journal of Chromatography A*, **1158(1-2)**, 168-173, 2007.
[10] McCalley D V. Effect of temperature and flow-rate on analysis of basic compounds in high-performance liquid chromatography using a reversed-phase column[J]. *Journal of Chromatography A*, **902(2)**, 311-321, 2000.
[11] Fu Q, Yang L M, Wang Q Q. Development of a portable micro-liquid chromatograph[J]. *Chromatograph*, **39(09)**, 1030-1037, 2021.
[12] Yang S D, Li N J, Ma Z, et al. Research advance in nano liquid chromatography instrumentation[J]. *Chromatograph*, **39(10)**, 1065-1076, 2021.
[13] Boswell P G, Schellenberg J R, Carr P W, et al. Easy and accurate high-performance liquid chromatography retention prediction with different gradients, flow rates, and instruments by back-calculation of gradient and flow rate profiles[J]. *Journal of Chromatography A*, **1218(38)**, 6742-6749, 2011.
[14] Liu H, Finch J W, Lavallee M J, et al. Effects of column length, particle size, gradient length and flow rate on peak capacity of nano-scale liquid chromatography for peptide separations[J]. *Journal of Chromatography A*, **1147(1)**, 30-36, 2007.
[15] Martin M, Guiochon G. Effects of high pressure in liquid chromatography[J]. *Journal of Chromatography A*, **1090(1-2)**, 16-38, 2005.
[16] Guillarme D, Ruta J, Rudaz S, et al. New trends in fast and high-resolution liquid chromatography: a critical comparison of existing approaches[J]. *Analytical and bioanalytical chemistry*, **397(3)**, 1069-1082, 2010.